

**Translation of letter from Ciba to the EPO, dated 4 September 2008**

**Opposition against European Patent No. 1 041 879**

**Application No.: 98954966.2**

**Proprietor: Pure Bioscience**

**El Cajon, CA 92020 (US)**

Our Ref: HM/1-E 3348

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An

**OPPOSITION**

according to Art. 99 EPC is hereby filed against European Patent No. 1 041 879 of the company PURE BIOSCIENCE with the title "Disinfectant and method of making", and

1. it is requested that the patent be revoked in its entirety in accordance with Art. 100 a) and 100 b) EPC, as its subject-matter is not novel and is not based on an inventive step (Art. 52 (1), Art. 41 (1) to (3), Art. 56 EPC) and the invention is not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 83 EPC), and

2. if the patent has not already been revoked in its entirety in the written proceedings, it is additionally requested that oral proceedings (Art. 116 EPC) be arranged.

The opposition fee is to be debited from our current account number 28110003 with the European Patent Organisation. A corresponding debit order is enclosed.

The following reference material is drawn on to substantiate the Opposition:

D1 US 4 915 955

D2 JP-A-07165510 (as Derwent Abstract No. 95-261152/34; a translation of JP-A-07165510 is submitted)

- D3 Krishna C. Mathur and Arun K. Dey, "On the composition of silver citrate", Jour. Indian Chem. Soc. Vol. 84, No. 9, 1957, page 713
- D4 US 4 755 268
- D5 Srivastava G. C. et al., "Development of ready to use antiseptic dressings", Labdev J. Sci. Tech., Vol. 8-B, No. 4, October 1970, pages 209 to 213
- D6 WO 96/39837
- D7 The Merck Index, Twelfth Edition, 1996, page 392

### **1. Insufficient disclosure, Article 83 EPC (Article 100(b) EPC)**

As regards the amounts of silver citrate specified in percent by volume in claims 7, 10, 18 and 30, it is left unclear to the person skilled in the art what amounts are to be used. Silver citrate is a substance which is present in solid form at room temperature and which is present in solution in the aqueous formulation. This leads to the question of how the specified amount in percent by volume is to be understood. This specified amount could possibly be interpreted, for example, as being a volume which is calculated from the density of the silver citrate, or equally as being the increase in the volume of the corresponding solution caused by the presence of silver citrate.

This applies analogously to claims 8, 9, 14, 15 and 19, in which an amount is specified in percent by volume, albeit only for silver in these cases.

As regards the specified amounts of citric acid, it is assumed that these are percentages by volume throughout. This results in similar ambiguities, because citric acid is also a substance which is in solid form at room temperature.

### **2. Novelty, Article 54 EPC (Article 100(a) EPC)**

The independent claim 1 of the disputed patent relates to an aqueous disinfectant, comprising: an aqueous solution of silver citrate wherein the silver ions are electrolytically generated in a solution of citric acid and water to form a solution comprising

1 % to 10 % citric acid and

a complex having the formula  $\text{Ag}^+\text{CA}^-$ , wherein  $\text{CA}^-$  is the citric acid anion.

The dependent claims 2 to 5 relate to specifications as to the metal complexes formed by the silver with the citric acid.

The independent claims 6 to 9 basically relate to preferred concentrations for the citric acid and/or the electrolytically generated silver or the silver citrate formed thereby. Claims 8 to 9 also relate to concentrated forms of the disinfectant having an extended shelf life.

The independent claim 10 of the disputed patent relates to an aqueous disinfectant comprising:

an aqueous solution of silver citrate in a solution of citric acid and water wherein

the concentration of silver citrate exceeds 0.05 % by volume comprising

1 % to 10 % citric acid and

a complex having the formula  $\text{Ag}^+\text{CA}^-$ , wherein

$(\text{CA})^-$  is the citric acid anion.

The independent claim 11 of the disputed patent relates to an aqueous disinfectant as defined in claim 10, wherein

the aqueous solution of silver citrate is formed from electrolytically generated silver in a solution of citric acid and water, and

the disinfectant further comprises approximately 20 % alcohol by volume.

According to the dependent claim 12, the alcohol is ethyl alcohol.

The dependent claims 13 to 15 basically relate to preferred concentrations for the citric acid and/or the electrolytically generated silver.

The independent claim 16 of the disputed patent relates to an aqueous disinfectant, comprising:

an aqueous solution of silver citrate wherein silver ions are electrolytically generated in a solution of citric acid and water to form a solution comprising  
1 % to 10 % citric acid and  
a complex having the formula  $\text{Ag}^+\text{CA}^-$ , wherein  
( $\text{CA}^-$ ) is the citric acid anion,  
approximately 20 % ethyl alcohol by volume, and  
approximately 0.01 % to 0.1 % anionic detergent by volume.

The independent claims 17 to 19 basically relate to preferred concentrations for the citric acid and/or the electrolytically generated silver or the silver citrate formed thereby. The independent claim 20 specifies that the detergent is sodium dodecylsulphate.

The independent claim 21 of the disputed patent relates to a process for making a disinfectant, wherein  
silver is electrolytically generated in a solution of citric acid and water to form an aqueous solution of silver citrate comprising  
1 % to 10 % citric acid and  
a complex having the formula  $\text{Ag}^+\text{CA}^-$  wherein  
( $\text{CA}^-$ ) is the citric acid anion.

The dependent claims 22 to 29 relate to preferred embodiments of this process.

The independent claim 30 of the disputed patent relates to an aqueous solution of silver citrate, comprising:  
an aqueous solution of silver citrate in a solution of citric acid and water comprising  
1 % to 10 % citric acid and  
a complex having the formula  $\text{Ag}^+\text{CA}^-$ , wherein ( $\text{CA}^-$ ) is the citric acid anion and wherein  
the concentration of silver citrate exceeds 0.05 % by volume.

The claimed compositions and the compositions obtained by the claimed processes are basically intended to be used as disinfectants, which have, *inter alia*, an improved shelf life.

In the following discussion of the state of the art, in the claims relating to the compositions, insofar as it is specified there, the feature that the silver ions are produced electrolytically in a solution of citric acid and water is not considered to be a feature differentiating from the state of the art. It is not discernable how compositions obtained in this manner differ from those which are obtained in another way, such as by reacting a silver salt with citric acid to form complexes of the formula  $\text{Ag}^+\text{CA}^-$ .

Column 12, lines 32 to 42 of the disputed patent refer to a higher solubility as compared with other silver citrates. The solubility cited there from the Merck Index, 11<sup>th</sup> Edition (1989), page 1348 refers to compounds of the formula  $\text{C}_6\text{H}_5\text{Ag}_3\text{O}_7$ , and not to those of the formula  $\text{Ag}^+\text{CA}^-$ . This comparison therefore cannot provide any indication as to whether electrolytically obtained compounds of the formula  $\text{Ag}^+\text{CA}^-$  differ in solubility from compounds of the formula  $\text{Ag}^+\text{CA}^-$  obtained in other ways. For completeness' sake, it should be noted that silver citrates with a better solubility than compounds of the formula  $\text{C}_6\text{H}_5\text{Ag}_3\text{O}_7$  had already been disclosed in D3 in 1957 (see the following discussion of D3), and in D5 in 1970. Thus, in the right-hand column on page 209 of D5, an aqueous solution containing 0.5 % silver citrate is disclosed, and this has a greater solubility than the solubility specified in the Merck Index for the compound of formula  $\text{C}_6\text{H}_5\text{Ag}_3\text{O}_7$ .

Corresponding compositions according to the disputed patent are already known from D1, D2 and D3.

a) Example 1, column 4, line 62 to column 5, line 21 of **D1** discloses the production of a solution which contains

1 litre of water,

100 g 75 % aqueous phosphoric acid,

140 g silver nitrate,

50 g citric acid, and

20 g gelatin.

The solution obtained has a pH of less than or equal to 1.6.

According to D1, the concentrates disclosed therein have an unlimited shelf-life (see the abstract on page 1 of D1) and are used as disinfectants.

The amount of 50 g citric acid used in Example 1 corresponds to an amount of approximately 3.8 % by weight, based on the total weight of the solution. Further, the 50 g of citric acid used in the Example correspond to  $30 \text{ cm}^3$  (at a citric acid density of  $1.665 \text{ g/cm}^3$ ).

It is therefore to be assumed that the citric acid is present in an amount of more than 1 % by volume based on the total volume of the solution.

As regards the presence of complexes of the formula  $\text{Ag}^+\text{CA}^-$ , it should be noted that these are already present due to the dissociation of the citric acid which takes place in the aqueous solution and due to the presence of the silver nitrate salt.

The citric acid is a multiprotic acid. At low pHs, such as that of less than or equal to 1.6 in the Example of D1, the further deprotonation of a  $\text{CA}^-$  radical is reduced in such a way that the  $\text{CA}^-$  radical is still obtained at relatively high concentrations (see also the pK values of the individual dissociation stages of the citric acid:  $\text{pK}_1 = 3.128$ ,  $\text{pK}_2 = 4.761$ ,  $\text{pK}_3 = 6.396$  according to D7). It is therefore to be assumed that in Example 1 of D1, the  $\text{CA}^-$  radical, and also the complex of formula  $\text{Ag}^+\text{CA}^-$  due to the introduced silver nitrate, are present at a relatively high concentration.

As regards the dependent claims 2 to 5, it is to be noted that the formation of the complexes mentioned therein is inherently connected with the production of corresponding solutions.

D1 is therefore prejudicial to the novelty of the subject-matters of claims 1 to 5, 8, 10 and 30 of the disputed patent.

b) In the Derwent Abstract Example, **D2** discloses the production of a solution containing  
10 g chitosan,  
1,000 ml water,  
10 g citric acid, and

500 mg silver nitrate.

It is specified that the citric acid is preferably used in a concentration of 0.1 to 10 %.

It is specified that these are disinfectants and have a good shelf-life.

When using 10 % citric acid, as proposed in the description, the Example of D2 results in an amount of 10 % by volume or 10 % by weight citric acid (since the percentage is not specified in greater detail in D2). Even making the assumption that this amount is being specified in % by weight, this results in an amount greater than 5 % by volume, taking into account the density of the citric acid.

As regards the presence of complexes of formula  $\text{Ag}^+\text{CA}^-$ , reference is made to the arguments given above in the discussion of D1. Therefore, these complexes also ought to be present in the Example of D2.

D2 is therefore also prejudicial to the novelty of the subject-matters of claims 1 to 7 and 30 of the disputed patent.

c) The fourth paragraph of **D3** discloses the production of a mixture of equal volumes of a 0.125-molar aqueous silver nitrate solution and a 1-molar aqueous citric acid solution.

This results in a citric acid content of approximately 9.6 % by weight. Taking into account the density of the citric acid, this results in an amount greater than 5 % by volume.

It is further disclosed that when sodium, potassium or ammonium hydroxide is added to the mixture,  $\text{Ag}_3$  citrate is precipitated. In this regard, it is disclosed that a soluble silver citrate is unstable in the presence of alkali and is precipitated as silver citrate ( $\text{Ag}_3$  citrate) when hydroxyl ions are added.

Therefore, in the specified mixture, a soluble silver citrate is obtained and precipitates as  $\text{Ag}_3$  citrate in the alkaline range. Due to the solubility in the relatively low pH range, the silver

citrate should be in the form of the  $\text{Ag}^+\text{CA}^-$  complex (see also the above arguments relating to D1, regarding the formation of the  $\text{Ag}^+\text{CA}^-$  complex at low pHs).

It should also be noted that it is disclosed in the first paragraph of D3 that silver citrate can be dissolved in an excess of citric acid.

Based on the amount of silver nitrate used and on the above arguments, this complex should be present in amounts greater than 0.05 % by volume.

The use of silver as a disinfectant has long been known and is part of general specialised knowledge.

D3 is therefore prejudicial to the novelty of the subject-matters of claims 1 to 10 and 30 of the disputed patent.

### **3. Inventive step, Article 56 EPC (Article 100(a) EPC)**

a) D1 or combination of D1 with D4

D1 relates to aqueous, storage-stable, clear concentrates, containing an inorganic acid for yielding a pH of less than or equal to 1.6, a silver salt or a silver salt complex, and an organic acid as a stabiliser (see for example claim 1 of D1).

According to column 2, lines 63 and 64 of D1, tartaric acid and/or citric acid are preferred as stabilisers. The stabilising effect of citric acid is also known from D6 (see D6, page 1, lines 16 to 20).

The above concentrates may be mixed with hydrogen peroxide, resulting in a synergy of the two disinfectants (i.e. between the concentrate containing silver salt and the hydrogen peroxide (see column 1, line 65 to column 2, line 4 of D1)).



Example 1 of D1 already discloses solutions in accordance with the disputed patent.

Claims 6, 7, 9, and 11 to 20 merely additionally specify the amount of citric acid and/or silver citrate or silver, or relate to the additional presence of approximately 20 % of an alcohol (in particular ethanol) and approximately 0.01 to 0.1 % of an anionic detergent (in particular sodium dodecylsulphate).

The amounts of the components mentioned are already routinely varied by the person skilled in the art; the alcohol and the anionic detergents are conventional additives, and so these embodiments cannot form the basis for the presence of an inventive step.

It should further be noted that electrolytic methods for the production of disinfectants containing silver have long been known (see D4).

Thus, D4 discloses a conventionally used method for the production of water containing silver ions, according to which the silver ions are produced by applying a voltage between an anode provided with silver and a cathode (see column 1, lines 46 to 52 of D4). The method claimed in D4 also makes use of this procedure (see the abstract on the title page of D4).

The electrolytic production of silver ions is therefore a conventional method, which the person skilled in the art would also consider suitable for the production of concentrates such as are disclosed in D1.

The method according to claim 21 of the disputed patent thus differs from the method disclosed in Example 1 of D1 only as regards the electrolytic production of the silver ions in the solution of citric acid and water. This procedure is made obvious by the combination of D1 and D4.

As regards the method claims 22 to 25, it should be noted that the formation of the complexes mentioned there is inherently connected with the production of corresponding solutions.

Method claims 26 to 29 relate to conventional embodiments of the electrolytic method.

Claims 1 to 30 of the disputed patent are therefore, insofar as they are not already anticipated in a manner prejudicial as to novelty, made obvious by D1 or the combination of D1 with D4.

b) D2 or combination of D2 with D4

D2 relates to aqueous disinfectants, comprising inorganic and/or organic acids, silver compounds (preferably silver nitrate) and chitosan.

Citric acid or acetic acid in a concentration of 0.1 to 10 % is preferred as an organic acid.

It is further disclosed that these solutions have a good shelf-life (for example two years at room temperature).

The Example of D2 already discloses solutions according to the disputed patent.

For the reasons given above under 3a), claims 1 to 30 of the disputed patent are therefore, insofar as they are not already anticipated in a manner prejudicial as to novelty, also made obvious by D2 or the combination of D2 with D4 in an analogous manner.

c) D3 or combination of D3 with D4

The fourth paragraph of D3 discloses the production of a mixture of equal volumes of a 0.125-molar aqueous silver nitrate solution and a 1-molar aqueous citric acid solution.

Taking into account the density of the citric acid, this results in an amount of greater than 5 % by volume for the citric acid.

A soluble silver citrate is also described, and given the solubility, this must be the complex  $\text{Ag}^+\text{CA}^-$ .

Because of the amount of silver nitrate used, this complex ought to be present in amounts greater than 0.05 % by volume.

The use of silver as a disinfectant has long been known and is part of general specialised knowledge. Therefore, in particular on the basis of the disclosed solubility of the silver citrate, the person skilled in the art will consider solutions of this type to be suitable for the production of disinfectants.

For the reasons already described above under 3a), claims 1 to 30 of the disputed patent are therefore, insofar as they are not already anticipated in a manner prejudicial as to novelty, also made obvious by D3 or a combination of D3 and D4 in an analogous manner.

4. To summarise, we maintain that none of the claims of European Patent No. 1 041 879 meets the requirements for patentability. On the basis of the above arguments, the request made at the outset for complete revocation of European Patent No. 1 041 879 is therefore justified and grounded.

Yours faithfully,  
Ciba Holding Inc.

[--Signed--]

Dr. Manfred Upschulte  
European Patent Attorney

[--Signed--]

Dr. Richard Schumacher  
European Patent Attorney

Enclosures:

Debit order for the Opposition fee

D1 [ = US 4 915 955], in duplicate

D2 [ = JP-A-07165510 (as Derwent Abstract No. 95-261152/34), in duplicate

D3 [ = Krishna C. Mathur and Arun K. Dey, "On the composition of silver citrate", Jour. Indian Chem. Soc. Vol. 84, No. 9, 1957, page 713], in duplicate

D4 [ = US 4 755 268], in duplicate

D5 [= Srivastava G. C. et al., "Development of ready to use antiseptic dressings", Labdev J. Sci. Tech., Vol. 8-B, No. 4, October 1970, pages 209 to 213], in duplicate

D6 [= WO 96/39837], in duplicate

D7 [= The Merck Index, Twelfth Edition, 1996, page 392], in duplicate

Copy for the other party

Documents D1 to D7 and copy for the other party only with letter of confirmation

**Translation of letter from Ciba to the European Patent Office, dated 4 September 2008**

Dear Sirs,

**Debit order: Opposition fee**

Internal Opposition Number: HM/1-E 3348

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010 ☒ The opposition fee of 670.00 Euros for the appended opposition against  
European Patent 1 041 879 in the name of PURE BIOSCIENCE

is to be debited from our current account number 2811 0003 with the European Patent  
Organisation in Munich upon the receipt of this payment form.

If the fee specified is incorrect, the European Patent Office is authorised to debit the correct  
amount.

Yours faithfully,  
Ciba Holding Inc.

[--Signed--]  
Dr. Manfred Upschulte  
European Patent Attorney

[--Signed--]  
Dr. Richard Schumacher  
European Patent Attorney

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CONFIRMATION



European Patent Office

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Our Ref: AMB/MS269043EP/KAS

Your Ref:

BY FAX

Date: 1 July 2009

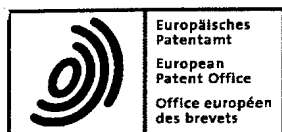
Dear Sirs

**Opposition to EP 1041879 (Pure Bioscience) by Ciba Holding Inc., Switzerland**

Further to the communication dated 27 April 2009, if, pursuant to proceedings under Article 101 EPC, the Opposition Division is not minded to uphold the patent, as granted, oral proceedings are hereby requested prior to the issuance of any decision that may adversely affect the applicant.

Yours faithfully

**Alan Bhimani**  
for MARKS & CLERK LLP

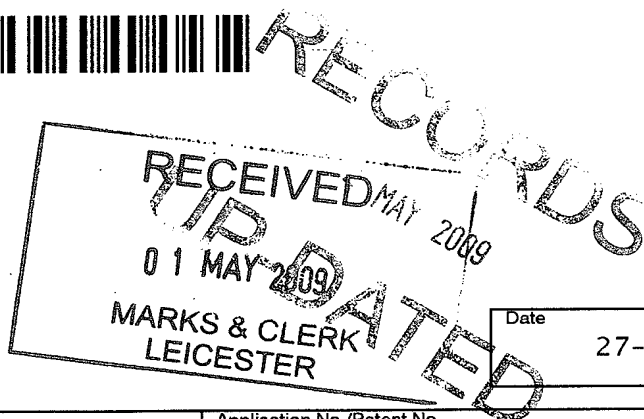


# NS 269013 EP

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Date 27-04-2009

Reference DCS/APH/P01396E	Application No./Patent No. 98954966.2 - 2103 / 1041879
Applicant/Proprietor Pure Bioscience	

**Failure to respond to the communication pursuant to Rule 79(1) EPC dated 13.11.08**

Failure to reply to the communication mentioned above does not lead to termination of the opposition proceedings.

In order to foreshorten the procedure and avoid unnecessary effort, we advise you of the following possibility if you are no longer interested in the maintenance of the above-mentioned patent.

**You have an opportunity before the European Patent Office to request that the patent be revoked or to state that you no longer approve the text in which the patent was granted.**

The result of the revocation decision is that the European patent is deemed not to have had, as from the outset, the effects specified in Articles 64 and 67 EPC (Art. 68 EPC).

If, within the period indicated below, you do not request the revocation of the patent or state as above that you no longer approve the text in which the patent was granted and if the prerequisites for the procedure pursuant to Rule 84(1) EPC are not fulfilled within that period, it is likely that the opposition proceedings will be continued pursuant to Article 101 EPC.

Any action on your part should be taken within **two months** of notification of this communication.

**For the Opposition Division**



## Marks&Clerk

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Reviewed *[Signature]* Name/Date  
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BY EMAIL

Our Ref: AMB/A16831/KAS  
Your Ref: 16200.0006EP1

**SCANNED**

19 December, 2008

Dear Mr Hathcock

### European Patent Application Number GB 1041879

Title: Opposition to EP 1041879 (Pure Bioscience) by Ciba Holding Inc., Switzerland

Proprietor(s): Pure Bioscience

We have recently received the attached communication from the European Patent Office forwarding to us a letter from the opponent. We are pleased to inform you that the opposition to the above patent has been withdrawn in its entirety. As such, there is no need for us to file written observations in response to the documentation filed by the opponent.

I should inform you that, pursuant to Rule 84 (2) EPC, the European Patent Office may continue opposition proceedings of its own motion when an opposition is withdrawn. However, this usually only occurs when either the proprietor has filed a set of amended claims, which in this case we have not, or where the European Patent Office anticipates that the patent is likely to be revoked in its entirety. As such, I would be surprised if, in this case, the European Patent Office were to pursue such a course of action.

I recommend that you study the opposition and the prior art cited and determine in your own mind if there are any valid points raised about the validity of the application. If so you may wish to consider making an amendment to the claims of the granted patent as, in some countries within Europe, if an attempt were made to bring proceedings on a patent that is known not to be valid, it may prejudice any chance there may be of success. Such an amendment to the claims is possible as a central process within the European Patent Office and, even though the patent is now granted, need not be done independently in each State.

If you are interested in pursuing such a course of action, please let me know and I can provide an estimate of the costs associated therewith.

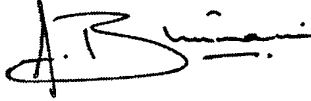
We will, of course, keep you up-dated with any future events relating to this issue and, if by chance the European Patent Office did wish to proceed with the opposition then we will receive a communication from them stating their intention to do so.

E-CLIEN.Doc



In the meantime, please find enclosed herewith an invoice for our services to-date for this matter.

Yours sincerely

A handwritten signature in black ink, appearing to read 'A. Bhimani', with a stylized flourish at the end.

Alan Bhimani  
Marks & Clerk  
E-mail: [abhimani@marks-clerk.com](mailto:abhimani@marks-clerk.com)



A168-1

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RECEIVED

12 SEP 2008

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Date

10-09-2008

Reference DCS/APH/P01396E	Application No./Patent No. 98954966.2 - 2103 / 1041879
Applicant/Proprietor Pure Bioscience	

**Communication of a notice of opposition**

Enclosed herewith is a copy of a notice of opposition to the European patent specified above.

An invitation to file observations and to file amendments, where appropriate, to the description, claims and drawings (R. 79(1) EPC) will be issued separately.

The period within which such observations may be filed will not be fixed until the following conditions are met:

- (a) the opposition period has expired;
- (b) the notice of opposition has been examined for certain formal requirements (R. 77 EPC).

Enclosure: Notice of opposition      OI: Ciba Holding Inc. (fax)

**For the Opposition Division**



Group Service  
Law & Environment  
Intellectual Property

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EPO - Munich  
31

05. Sep. 2008

CONFIRMATION COPY

**Ciba**



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TELEFAX

FaxNo: +4989 2399 4465

Pages: 11

4. September 2008

**Einspruch gegen das Europäische Patent Nr. 1 041 879**

**Anmeldenummer: 98954966.2**

**Eigentümer: Pure Bioscience  
El Cajon, CA 92020 (US)**

Unser Zeichen: HM/1-E 3348

Gegen das Europäische Patent Nr. 1 041 879 der Firma PURE BIOSCIENCE mit dem Titel „Disinfectant and method of making“ wird

### EINSPRUCH

**Zur Kasse**  
670. FrA

gemäss Art. 99 EPUe erhoben und

1. beantragt, es aufgrund von Art. 100 a) und 100 b) EPUe in vollem Umfang zu widerrufen, da sein Gegenstand nicht neu ist und nicht auf einer erfinderischen Tätigkeit beruht (Art. 52(1), Art. 54 (1) bis (3), Art. 56 EPUe); und die Erfindung nicht so deutlich und vollständig offenbart ist, dass ein Fachmann sie ausführen kann (Art. 83 EPUe), und
2. für den Fall, dass das Patent nicht bereits im schriftlichen Verfahren in vollem Umfang widerrufen wird, hilfsweise beantragt, eine mündliche Verhandlung (Art. 116 EPUe) anzuberaumen.

Die Einspruchsgebühr ist von unserem laufenden Konto Nr. 28110003 bei der Europäischen Patentorganisation abzubuchen. Ein entsprechender Abbuchungsauftrag liegt bei.

Zur Substantiierung des Einspruchs wird folgendes Beweismaterial herangezogen:

- D1 US 4 915 955
- D2 JP-A-07165510 (als Derwent Abstract Nr. 95-261152/34; eine Übersetzung der JP-A-07165510 wird nachgereicht)
- D3 Krishna C. Mathur and Arun K. Dey, "On the composition of silver citrate", Jour. Indian Chem. Soc. Vol. 84, No. 9, 1957, Seite 713
- D4 US 4 755 268
- D5 Srivastava G.C. et al., "Development of ready to use antiseptic dressings", Labdev J. Sci. Tech., Vol. 8-B, No. 4, October 1970, Seiten 209 bis 213
- D6 WO 96/39837
- D7 The Merck Index, Twelfth Edition, 1996, Seite 392

### 1. Mangelnde Offenbarung Artikel 83 EPÜe (Artikel 100(b) EPÜe)

Hinsichtlich der in den Ansprüchen 7, 10, 18 und 30 angegebenen Mengenangabe des Silbercitrats in Volumenprozent bleibt es für den Fachmann unklar, welche Mengen zu verwenden sind. So handelt es sich im Falle des Silbercitrats um eine bei Raumtemperatur in fester Form vorliegende Substanz, die in der wässrigen Formulierung gelöst vorliegt. Es besteht daher die Frage, wie die Mengenangabe in Volumenprozent zu verstehen ist. Diese Mengenangabe könnte u.U. so interpretiert werden, dass es sich z.B. um ein Volumen handelt, welches sich aus der Dichte des Silbercitrats errechnet, oder auch um die Volumenzunahme der entsprechenden Lösung, welche durch die Gegenwart von Silbercitrat hervorgerufen wird.

Dies gilt in analoger Weise für die Ansprüche 8, 9, 14, 15 und 19, worin eine Mengenangabe in Volumenprozent, in diesen Fällen jedoch nur für Silber, erfolgt.

Hinsichtlich der Mengenangaben für die Citronensäure wird angenommen, dass es sich hierbei durchgehend um Volumenprozent handeln soll. Es bestehen daher gleichermassen Unklarheiten, da auch Citronensäure eine bei Raumtemperatur in fester Form vorliegende Substanz ist.

### 2. Neuheit Artikel 54 EPÜ (Artikel 100(a) EPÜ)

Der unabhängige Anspruch 1 des Streitpatents ist auf ein wässriges Desinfektionsmittel gerichtet, umfassend

eine wässrige Silbercitrat-Lösung, wobei die Silberionen elektrolytisch in einer Lösung aus Citronensäure und Wasser erzeugt werden, um eine Lösung zu bilden, die

1 bis 10% Citronensäure und einen Komplex der Formel  $\text{Ag}^+\text{CA}^-$  umfasst, wobei  $\text{CA}^-$  das Citronensäureanion ist.

Die abhängigen Ansprüche 2 bis 5 betreffen Spezifizierungen bezüglich der vom Silber mit der Citronensäure gebildeten Metallkomplexe.

Die abhängigen Ansprüche 6 bis 9 betreffen im Wesentlichen bevorzugte Konzentrationen für die Citronensäure und/oder das elektrolytisch erzeugte Silber bzw. das mit diesem gebildete Silbercitrat. Die Ansprüche 8 und 9 sind zudem auf konzentrierte Formen des Desinfektionsmittels mit einer verlängerten Lagerstabilität gerichtet.

Der unabhängige Anspruch 10 des Streitpatents ist auf ein wässriges Desinfektionsmittel gerichtet, umfassend eine wässrige Silbercitrat-Lösung in einer Lösung aus Citronensäure und Wasser, wobei die Silbercitrat-Konzentration mehr als 0,05 Vol.-% beträgt, umfassend 1 bis 10% Citronensäure und einen Komplex der Formel  $\text{Ag}^+\text{CA}^-$ , wobei  $\text{CA}^-$  das Citronensäureanion ist.

Der unabhängige Anspruch 11 des Streitpatents ist auf ein wässriges Desinfektionsmittel wie in Anspruch 10 definiert gerichtet, wobei die wässrige Silbercitrat-Lösung aus elektrolytisch erzeugtem Silber in einer Lösung aus Citronensäure und Wasser gebildet ist, und das Desinfektionsmittel ferner etwa 20 Vol.-% Alkohol umfasst.

Gemäss dem abhängigen Anspruch 12 handelt es sich bei dem Alkohol um Ethylalkohol.

Die abhängigen Ansprüche 13 bis 15 betreffen im Wesentlichen bevorzugte Konzentrationen für die Citronensäure und/oder das elektrolytisch erzeugte Silber.

Der unabhängige Anspruch 16 des Streitpatents ist auf ein wässriges Desinfektionsmittel gerichtet, umfassend eine wässrige Silbercitrat-Lösung, wobei die Silberionen elektrolytisch in einer Lösung aus Citronensäure und Wasser erzeugt werden, zur Bildung einer Lösung umfassend 1 bis 10% Citronensäure und einen Komplex der Formel  $\text{Ag}^+\text{CA}^-$ , wobei  $\text{CA}^-$  das Citronensäureanion ist, etwa 20 Vol.-% Ethylalkohol, und etwa 0,01 Vol.-% bis 0,1 Vol.-% anionisches Detergens.

Die abhängigen Ansprüche 17 bis 19 betreffen im Wesentlichen bevorzugte Konzentrationen für die Citronensäure und/oder das elektrolytisch erzeugte Silber bzw. das mit diesem gebildete Silbercitrat. Im abhängigen Anspruch 20 ist das Detergens als Natriumdodecylsulfat spezifiziert.

Der unabhängige Anspruch 21 des Streitpatents ist auf ein Verfahren zur Herstellung eines Desinfektionsmittel gerichtet, worin Silber elektrolytisch in einer Lösung aus Citronensäure und Wasser erzeugt wird zur Bildung einer wässrigen Silbercitrat-Lösung umfassend 1 bis 10% Citronensäure und einen Komplex der Formel  $\text{Ag}^+\text{CA}^-$ , wobei  $\text{CA}^-$  das Citronensäureanion ist.

Die abhängigen Ansprüche 22 bis 29 betreffen bevorzugte Ausführungsformen dieses Verfahrens.

Der unabhängige Anspruch 30 des Streitpatents ist auf eine wässrige Silbercitrat-Lösung gerichtet, umfassend eine wässrige Silbercitrat-Lösung in einer Lösung aus Citronensäure und Wasser umfassend 1 bis 10% Citronensäure und einen Komplex der Formel  $\text{Ag}^+\text{CA}^-$  wobei  $\text{CA}^-$  das Citronensäureanion ist, und wobei die Silbercitratkonzentration 0,05 Vol.-% übersteigt.

Im Wesentlichen sollen die beanspruchten Zusammensetzungen bzw. die gemäss den beanspruchten Verfahren erhaltenen Zusammensetzungen als Desinfektionsmittel eingesetzt werden, welche u.a. eine verbesserte Lagerstabilität aufweisen.

In der folgenden Diskussion des Standes der Technik wird in den die Zusammensetzungen betreffenden Ansprüchen, soweit dort angegeben, das Merkmal, dass die Silberionen elektrolytisch in einer Lösung aus Citronensäure und Wasser erzeugt werden, nicht als Unterscheidungsmerkmal zum Stand der Technik betrachtet. Es ist nicht erkennbar, wie sich auf diese Weise erhaltene Zusammensetzungen von solchen unterscheiden, die auf andere Art erhalten werden, wie z.B. durch Reaktion eines Silbersalzes mit Citronensäure unter Bildung von Komplexen der Formel  $\text{Ag}^+\text{CA}^-$ .

In Spalte 12, Zeilen 32 bis 42 des Streitpatents wird auf eine erhöhte Löslichkeit gegenüber anderen Silbercitraten verwiesen. Die dort aus dem Merck Index, 11. Ausgabe (1989), Seite 1348 zitierte Löslichkeit betrifft jedoch Verbindungen der Formel  $\text{C}_6\text{H}_5\text{Ag}_3\text{O}_7$ , und nicht solche der Formel  $\text{Ag}^+\text{CA}^-$ . Dieser Vergleich kann daher keinerlei Hinweise darauf geben, ob sich die auf elektrolytischem Wege erhaltenen Verbindungen der Formel  $\text{Ag}^+\text{CA}^-$  hinsichtlich der Löslichkeit von auf anderen Wegen erhaltenen Verbindungen der Formel  $\text{Ag}^+\text{CA}^-$  unterscheiden. Der Vollständigkeit halber sei erwähnt, dass Silbercitrate mit einer gegenüber den Verbindungen der Formel  $\text{C}_6\text{H}_5\text{Ag}_3\text{O}_7$  besseren Löslichkeit bereits 1957 in der D3

(siehe hierzu die folgenden Ausführungen zur D3), sowie auch 1970 in der D5 offenbart sind. So ist in der rechten Spalte auf Seite 209 der D5 eine wässrige Lösung enthaltend 0,5% Silbercitrat beschrieben; diese Löslichkeit liegt oberhalb der im Merck Index für die Verbindung der Formel  $C_6H_5Ag_3O_7$  angegebenen Löslichkeit.

Entsprechende Zusammensetzungen gemäss Streitpatent sind bereits aus der D1, D2 und D3 bekannt.

a) D1 offenbart in Beispiel 1, Spalte 4, Zeile 62 bis Spalte 5, Zeile 21 die Herstellung einer Lösung, welche

1 Liter Wasser,

100 g 75 %-ige wässrige Phosphorsäure,

140 g Silbernitrat,

50 g Citronensäure, und

20 g Gelatine

enthält. Die erhaltene Lösung hat einen pH-Wert von kleiner oder gleich 1.6.

Gemäss der D1 weisen die dort beschriebenen Konzentrate eine unbegrenzte Lagerstabilität auf (siehe z.B. die Zusammenfassung auf Seite 1 der D1) und werden für Desinfektionsmittel verwendet.

Die in Beispiel 1 eingesetzte Menge von 50 g Citronensäure entspricht einer Menge von ca. 3,8 Gew.-%, bezogen auf das Gesamtgewicht der Lösung.

Ferner entsprechen die in dem Beispiel eingesetzten 50 g der Citronensäure 30 cm<sup>3</sup> (bei einer Dichte der Citronensäure von 1,665 g/cm<sup>3</sup>).

Es ist daher anzunehmen, dass die Citronensäure in einer Menge oberhalb von 1 Vol.-%, bezogen auf das Gesamtvolumen der Lösung, vorliegt.

Hinsichtlich der Gegenwart von Komplexen der Formel  $Ag^+CA^-$  ist anzumerken, dass diese schon bereits aufgrund der in wässriger Lösung stattfindenden Dissoziation der Citronensäure, sowie aufgrund der Gegenwart des Silbernitratsalzes, vorliegen.

Im Falle der Citronensäure handelt es sich um eine mehrprotonige Säure. Bei geringen pH-Werten, wie dem in dem Beispiel der D1 von kleiner oder gleich 1,6, wird die weitere Deprotonierung eines Restes  $CA^-$  umso geringer sein, so dass der Rest  $CA^-$  in höheren Konzentrationen erhalten bleibt (siehe auch die pK-Werte für die einzelnen Dissoziationsstufen der Citronensäure:  $pK_1 = 3,128$ ,  $pK_2 = 4,761$ ,  $pK_3 = 6,396$  gemäss der D7). Es ist daher anzunehmen, dass im Beispiel 1 der D1 der Rest  $CA^-$  und aufgrund des eingebrachten Silbernitrats auch der Komplex der Formel  $Ag^+CA^-$  in grösserer Konzentration vorliegt.

Im Falle der abhängigen Ansprüche 2 bis 5 ist anzumerken, dass die Bildung der dort genannten Komplexe mit der Herstellung entsprechender Lösungen inhärent verbunden ist.

Die Gegenstände der Ansprüche 1 bis 5, 8, 10 und 30 des Streitpatents sind daher durch die D1 neuheitsschädlich getroffen.

b) D2 offenbart im Beispiel des Derwent Abstracts die Herstellung einer Lösung, welche

10 g Chitosan,  
1000 ml Wasser,  
10 g Citronensäure, und  
500 mg Silbernitrat  
enthält.

Für die Citronensäure ist angegeben, dass diese bevorzugt in einer Konzentration von 0,1 bis 10% verwendet wird.

Es wird angegeben, dass es sich hierbei um Desinfektionsmittel handelt und diese eine gute Lagerstabilität aufweisen.

Im Falle der Verwendung von 10% Citronensäure, wie in der Beschreibung vorgeschlagen, ergibt sich im Beispiel der D2 eine Menge von 10 Vol.-% oder 10 Gew.-% Citronensäure (da die Prozentangabe in der D2 nicht näher spezifiziert ist). Selbst unter der Annahme, dass es sich hierbei um eine Angabe in Gew.-% handelt, ergibt sich unter Berücksichtigung der Dichte der Citronensäure eine Menge oberhalb von 5 Vol.-%.

Hinsichtlich der Gegenwart von Komplexen der Formel  $\text{Ag}^+\text{CA}^-$  wird auf die oben im Rahmen der Besprechung der D1 erfolgten Ausführungen verwiesen. Diese Komplexe sollten daher auch im Beispiel der D2 vorliegen.

Die Gegenstände der Ansprüche 1 bis 7 und 30 des Streitpatents sind daher durch die D2 neuheitsschädlich getroffen.

c) D3 beschreibt im vierten Absatz die Herstellung einer Mischung aus gleichen Volumen einer 0,125 molaren wässrigen Silbernitratlösung und einer 1 molaren wässrigen Citronensäurelösung.

Hieraus ergibt sich ein Gehalt an Citronensäure von ca. 9,6 Gew.-%. Unter Berücksichtigung der Dichte der Citronensäure ergibt sich eine Menge oberhalb von 5 Vol.-%.

Ferner wird beschrieben, dass bei Zugabe von Natrium-, Kalium- oder Ammoniumhydroxid zu der Mischung  $\text{Ag}_3\text{Citrat}$  ausfällt. In diesem Zusammenhang wird beschrieben, dass ein lösliches Argentocitrat instabil in der Gegenwart von Alkali ist und bei Zugabe von Hydroxylionen als Silbercitrat ( $\text{Ag}_3\text{Citrat}$ ) ausfällt.

Es ist daher in der angegebenen Mischung ein lösliches Argentocitrat enthalten, welches im alkalischen Bereich als  $\text{Ag}_3\text{Citrat}$  ausfällt. Aufgrund der Löslichkeit im eher unteren pH-Bereich sollte es sich im Falle des Argentocitrats um den



Komplex  $\text{Ag}^+\text{CA}^-$  handeln (siehe hierzu auch die obigen Ausführungen zur D1, welche die Bildung des Komplexes  $\text{Ag}^+\text{CA}^-$  bei niedrigen pH-Werten betreffen).

Zudem sei noch erwähnt, dass im ersten Absatz der D3 beschrieben wird, dass Silbercitrat in einem Überschuss von Citronensäure gelöst werden kann.

Aufgrund der Menge des eingesetzten Silbernitrats und der obigen Ausführungen sollte dieser Komplex in Mengen oberhalb von 0,05 Vol.-% vorliegen.

Die Verwendung von Silber als Desinfektionsmittel ist seit langem bekannt und Teil des allgemeinen Fachwissens.

Die Gegenstände der Ansprüche 1 bis 10 und 30 des Streitpatents sind daher durch die D3 neuheitsschädlich getroffen.

### 3. Erfinderische Tätigkeit Artikel 56 EPÜ (Artikel 100(a) EPÜ)

a) D1 bzw. Kombination der D1 mit der D4

Die D1 betrifft wässrige, lagerstabile, klare Konzentrate, welche eine anorganische Säure zur Einstellung eines pH-Wertes von kleiner oder gleich 1,6, ein Silbersalz oder einen Silbersalzkomplex, sowie eine organische Säure als Stabilisierungsmittel enthalten (siehe z.B. Anspruch 1 der D1).

Gemäss Spalte 2, Zeilen 63 und 64 der D1 sind Weinsäure und/oder Citronensäure als Stabilisierungsmittel bevorzugt. Die stabilisierende Wirkung von Citronensäure ist auch aus der D6 bekannt ist (siehe D6, Seite 1, Zeilen 16 bis 20).

Die obigen Konzentrate können mit Wasserstoffperoxid gemischt werden, woraus eine Synergie aus beiden Desinfektionsmitteln (d.h. dem Silbersalz enthaltenden Konzentrat und dem Wasserstoffperoxid) resultiert (siehe Spalte 1, Zeile 65 bis Spalte 2, Zeile 4 der D1).

Das Beispiel 1 der D1 beschreibt bereits Lösungen gemäss Streitpatent.

Die Ansprüche 6, 7, 9 und 11 bis 20 spezifizieren lediglich zusätzlich die Menge der Citronensäure und/oder des Silbercitrats bzw. Silbers, oder betreffen die zusätzliche Gegenwart von ca. 20% eines Alkohols (insbesondere Ethanol) sowie ca. 0,01 bis 0,1% eines anionischen Detergens (insbesondere Natriumdodecylsulfat).

Die Variation der Mengen der genannten Komponenten wird von dem Fachmann bereits routinemässig ausgeführt; im Falle des Alkohols und des anionischen Detergens handelt es sich um übliche Zusätze, so dass diese Ausführungsformen nicht geeignet sind, um das Vorliegen einer erfinderischen Tätigkeit zu begründen.

Ferner sei erwähnt, dass elektrolytische Verfahren zur Herstellung von Silber enthaltenden Desinfektionsmitteln seit langem bekannt sind (siehe hierzu die D4).

So wird in der D4 ein herkömmlich benutztes Verfahren zur Erzeugung von Silberionen enthaltendem Wasser beschrieben, gemäss welchem die Silberionen durch Anlegen einer Spannung zwischen einer mit Silber ausgerüsteten Anode und einer Kathode erzeugt werden (siehe Spalte 1, Zeilen 46 bis 52 der D4). Auch das gemäss der D4 beanspruchte Verfahren macht von dieser Vorgehensweise Gebrauch (siehe die Zusammenfassung auf der Titelseite der D4).

Es handelt sich im Falle der elektrolytischen Erzeugung von Silberionen daher um ein übliches Verfahren, welches der Fachmann auch für die Herstellung von Konzentraten, wie sie in der D1 beschrieben sind, als geeignet betrachten wird.

So unterscheidet sich das Verfahren gemäss Anspruch 21 des Streitpatents von dem in Beispiel 1 der D1 offenbarten Verfahren lediglich hinsichtlich der elektrolytischen Erzeugung der Silberionen in der Lösung von Citronensäure und Wasser. Diese Vorgehensweise ist durch die Kombination der D1 mit der D4 nahegelegt.

Im Falle der Verfahrensansprüche 22 bis 25 ist anzumerken, dass die Bildung der dort genannten Komplexe mit der Herstellung entsprechender Lösungen inhärent verbunden ist.

Die Verfahrensansprüche 26 bis 29 betreffen übliche Ausführungsformen des elektrolytischen Verfahrens.

Die Ansprüche 1 bis 30 des Streitpatents sind daher, insoweit sie nicht bereits neuheitsschädlich vorweggenommen sind, durch die D1 oder die Kombination der D1 mit der D4 nahegelegt.

b) D2 bzw. Kombination der D2 mit der D4

Die D2 betrifft wässrige Desinfektionsmittel, welche anorganische und/oder organische Säuren, Silberverbindungen (vorzugsweise Silbernitrat) und Chitosan enthalten.

Als organische Säuren sind Citronensäure oder Essigsäure in einer Konzentration von 0,1 bis 10% bevorzugt.

Ferner wird beschrieben, dass diese Lösungen eine gute Lagerstabilität aufweisen (z.B. zwei Jahre bei Raumtemperatur).

Das Beispiel der D2 beschreibt bereits Lösungen gemäss Streitpatent.

Aus den bereits oben unter 3a) beschriebenen Gründen sind die Ansprüche 1 bis 30 des Streitpatents daher auch in analoger Weise, insoweit sie nicht bereits neuheitsschädlich vorweggenommen sind, durch die D2 oder die Kombination der D2 mit der D4 nahegelegt.

c) D3 bzw. Kombination der D3 mit der D4

Die D3 beschreibt im vierten Absatz die Herstellung einer Mischung aus gleichen Volumen einer 0,125 molaren wässrigen Silbernitratlösung und einer 1 molaren wässrigen Citronensäurelösung.

Unter Berücksichtigung der Dichte der Citronensäure ergibt sich für diese eine Menge oberhalb von 5 Vol.-%.

Zudem wird ein lösliches Argentocitrat beschrieben, wobei es sich aufgrund der Löslichkeit um den Komplex  $\text{Ag}^+\text{CA}^-$  handeln sollte.

Aufgrund der Menge des eingesetzten Silbernitrats sollte dieser Komplex in Mengen oberhalb von 0,05 Vol.-% vorhanden sein.

Die Verwendung von Silber als Desinfektionsmittel ist seit langem bekannt und Teil des allgemeinen Fachwissens. Der Fachmann wird daher, insbesondere aufgrund der beschriebenen Löslichkeit des Argentocitrats, solche Lösungen als geeignet für die Herstellung von Desinfektionsmitteln betrachten.

Aus den bereits oben unter 3a) beschriebenen Gründen sind die Ansprüche 1 bis 30 des Streitpatents daher auch in analoger Weise, insoweit sie nicht bereits neuheitsschädlich vorweggenommen sind, durch die D3 oder die Kombination der D3 mit der D4 nahegelegt.

4. Zusammenfassend wird festgehalten, dass keiner der Patentansprüche des Europäischen Patents Nr. 1 041 879 die Voraussetzungen für die Patentfähigkeit erfüllt. Aufgrund der vorstehenden Ausführungen ist daher der eingangs gestellte Antrag auf vollständigen Widerruf des Europäischen Patents Nr. 1 041 879 gerechtfertigt und begründet.

Mit freundlichen Grüßen  
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Beilagen:

Abbuchungsauftrag für die Einspruchsgebühr

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D2 [= JP-A-07165510 (als Derwent Abstract Nr. 95-261152/34)], zweifach

- D3 [= Krishna C. Mathur and Arun K. Dey, "On the composition of silver citrate", Jour. Indian Chem. Soc. Vol. 84, No. 9, 1957, Seite 713], zweifach  
D4 [= US 4 755 268], zweifach  
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D6 [= WO 96/39837], zweifach  
D7 [= The Merck Index, Twelfth Edition, 1996, Seite 392], zweifach  
Parteienexemplar

Dokumente D1 bis D7 und Parteienexemplar nur mit Bestätigungsschreiben

# United States Patent [19]

## Gömöri

[11] Patent Number: **4,915,955**  
 [45] Date of Patent: **Apr. 10, 1990**

### [54] PROCESS FOR PREPARING A DISINFECTANT

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[21] Appl. No.: 904,055

[22] Filed: Sep. 5, 1986

### [30] Foreign Application Priority Data

Apr. 22, 1986 [CH] Switzerland ..... 1629/86

[51] Int. Cl.<sup>4</sup> ..... A01N 39/00; A01N 59/16

[52] U.S. Cl. .... 424/616; 424/618

[58] Field of Search ..... 424/132, 128, 130, 616, 424/618; 514/495

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Assistant Examiner—Kevin E. Weddington

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### [57] ABSTRACT

A concentrate with an unlimited shelf-life, which can be mixed with hydrogen peroxide at a ratio of 1:99 to 1:199 to become an effective disinfectant, is obtained when a viscous solution of inorganic acid, with a pH less than or equal to 1.6, is mixed with a silver salt compound or a colloidal silver compound at 50° to 60° C. The mixture is further combined at room temperature with other inorganic acid(s) to reach a total of 100 g inorganic acid(s) per liter of water; at room temperature, an organic acid stabilizer is added and the mixture is homogenized. The concentrate, during storage, remains homogeneous and crystal-clear.

12 Claims, No Drawings

4,915,955

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## PROCESS FOR PREPARING A DISINFECTANT

## FIELD OF THE INVENTION

The present invention relates to a process for the manufacture of a concentrate which can be mixed with hydrogen peroxide to form a disinfectant.

## INTRODUCTION

Disinfection, especially of water, foodstuffs and animal feeds as well as equipment, packages, containers and objects of all kinds, is a worldwide problem of importance in highly civilized nations and underdeveloped countries alike. It is therefore not surprising that intensive research has been ongoing in this area for decades, and that new products and methods for the non-therapeutic battle against infectious agents are constantly being developed.

Of the numerous known prior art disinfection methods, the following are exemplary.

The addition of halogens such as chlorine, bromine and iodine, or of halogen-releasing compositions, is popular due to the ease of manufacture, low price, and ready availability of the constituent chemicals, as well as for its reliable germicidal action. Disadvantages include the initial taste and odor of the halogen, the change in taste of the compositions mixed with it, the overall basic pH, the relatively short duration of the germicidal activity, and the concomitant temperature sensitivity of the resultant combinations. In addition, these substances are carcinogenic and mutagenic, cause skin irritations, and are corrosive to a variety of materials.

Ozone and chlorine dioxide combine to form an especially quick and effective disinfectant, but the resultant combination remains effective for only a short time. These combined agents can also be very dangerous in large doses. Furthermore, they display temperature sensitivity, tend to be carcinogenic and mutagenic, and require both careful handling and expensive auxiliary equipment. They are therefore suitable only for a very narrow area of application.

Copper salts, especially copper sulfate and copper chloride, are recommended as disinfectants that are odorless and which do not irritate mucous membranes, etc. These combinations, which produce only an average, briefly-sustained disinfecting rate and are known to be carcinogenic and mutagenic, especially the chloride salts, do combine readily with other substances.

Processes which are common, odorless, and have no adverse effects on health are the so-called Catadyne method and similar methods utilizing the germicidal effect of silver ions. This process is slow-working and has limited application due to its high cost.

Ultraviolet radiation, which does not introduce foreign matter into the substance to be disinfected, is a reliable source of germicide activity. The generation of ultraviolet radiation, however, requires both expensive equipment and the substantial costs commensurate with large electricity requirements.

Other commercially available products rely heavily on the powerful germicidal activity of hydrogen peroxide, but such compositions have limited utility due to their instability. These compositions include those which combine the well-known germicidal effects of silver with hydrogen peroxide, in the form of a stable agent, to cause a synergy of the two disinfecting agents.

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As a result of the present invention, it is now possible to make products of this last type, having substantially improved quality and utility, by means of a new process.

It is an object of the present invention to provide a method for manufacturing a clear, stable concentrate which can be mixed with hydrogen peroxide to form a disinfectant, and which contains silver, an inorganic acid and an organic stabilizer.

It is a further object of the present invention to provide a concentrate of that type as well as a process for the manufacture of a disinfectant from such a concentrate.

## SUMMARY OF THE INVENTION

The present invention is a stable concentrate, and a method of preparing it and using it, which contains a silver compound, an inorganic acid and an organic stabilizer. The concentrate is designed to be admixed with hydrogen peroxide to form a disinfectant.

Suitable silver compounds include silver nitrate ( $\text{AgNO}_3$ ), silver sulfate ( $\text{Ag}_2\text{SO}_4$ ), silver chloride ( $\text{AgCl}$ ), and the sodium/silver chloride complex having the formula  $\text{AgNaCl}_2$ . Additional suitable silver compounds are silver benzoate ( $\text{C}_6\text{H}_5\text{CO}_2\text{Ag}$ ), silver carbonate ( $\text{Ag}_2\text{CO}_3$ ), silver fluoride ( $\text{AgF}$ ), silver [I] oxide ( $\text{Ag}_2\text{O}$ ) and silver [II] oxide ( $\text{AgO}$ ). One or more silver compound(s) is/are added in such amounts that the concentrate contains  $100 \text{ g} \pm 5\%$  silver per liter of concentrate.

Colloidal silver compounds may also be used, in one-tenth of the concentration of grams Ag per liter of concentrate as described above. A suitable colloidal silver compound, available from Degussa AG, Zurich, contains 12 g per liter of silver in an aqueous solution of 5% by weight polyhydroxyl monocarboxylic acid.

Tartaric acid and/or citric acid are suitable stabilizers for use in combination with the silver compound(s). These acids should be added in the amount of approximately 50 g of one and/or the other per liter of concentrate.

Additional suitable organic acid stabilizers, to be used in stoichiometric amounts equivalent to amounts specified for incorporation of the tartaric and/or citric acids, include acetamidoacrylic acid ( $\text{H}_2\text{C}=\text{C}(\text{NHCOCH}_3)\text{CO}_2\text{H}$ ), acetamidohexanoic acid ( $\text{CH}_3\text{CONH}(\text{CH}_2)_5\text{CO}_2\text{H}$ ), acetylbutyric acid ( $\text{CH}_3\text{CO}(\text{CH}_2)_3\text{CO}_2\text{H}$ ), acrylic acid ( $\text{H}_2\text{C}=\text{CHCO}_2\text{H}$ ), adipic acid ( $\text{HO}_2\text{C}(\text{CH}_2)_4\text{CO}_2\text{H}$ ), maleic acid ( $\text{HO}_2\text{CCH}_2\text{CH}(\text{OH})\text{CO}_2\text{H}$ ), ethoxyacetic acid ( $\text{C}_2\text{H}_5\text{OCH}_2\text{CO}_2\text{H}$ ), formic acid ( $\text{HCO}_2\text{H}$ ), succinic acid ( $\text{HO}_2\text{CCH}_2\text{CH}_2\text{CO}_2\text{H}$ ), butyric acid ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), hexanoic acid ( $\text{CH}_3(\text{CH}_2)_4\text{CO}_2\text{H}$ ), hippuric acid ( $\text{C}_6\text{H}_5\text{CONHCH}_2\text{CO}_2\text{H}$ ), malonic acid ( $\text{HO}_2\text{CCH}_2\text{CO}_2\text{H}$ ), methanesulfonic acid ( $\text{CH}_3\text{SO}_3\text{H}$ ), lactic acid ( $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{H}$ ), caprylic acid ( $\text{CH}_3(\text{CH}_2)_6\text{CO}_2\text{H}$ ), oleic acid ( $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$ ), oxalic acid ( $\text{HO}_2\text{CCO}_2\text{H}$ ), salicylic acid ( $\text{HOC}_6\text{H}_4\text{CO}_2\text{H}$ ), and valeric acid  $\text{CH}_3(\text{CH}_2)_3\text{CO}_2\text{H}$ . Although each of the above organic acids is suitable for use with either the silver salt or the colloidal silver preparation, they are especially well suited for use with the various silver salts. Most preferred for use with the silver salt compounds are, however, the tartaric and/or citric acids.

Although each of the organic acid stabilizers listed above is suitable for use in combination with colloidal silver, the following are preferred for use, in amounts of approximately 100 g per liter of concentrate: acetoned-

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carboxylic acid ( $\text{HO}_2\text{CCH}_2\text{COCH}_2\text{CO}_2\text{H}$ ), acetox-ybenzoic acid ( $\text{CH}_3\text{CO}_2\text{C}_6\text{H}_4\text{CO}_2\text{H}$ ), ethoxybenzoic acid ( $\text{C}_2\text{H}_5\text{OC}_6\text{H}_4\text{CO}_2\text{H}$ ), ethylbenzoic acid ( $\text{C}_2\text{H}_5\text{C}_6\text{H}_4\text{CO}_2\text{H}$ ), aminobenzoic acid ( $\text{H}_2\text{NC}_6\text{H}_4\text{CO}_2\text{H}$ ), benzoic acid ( $\text{C}_6\text{H}_5\text{CO}_2\text{H}$ ), bromo-<sup>5</sup>benzoic acid ( $\text{BrC}_6\text{H}_4\text{CO}_2\text{H}$ ), bromosalicylic acid ( $\text{BrC}_6\text{H}_3\text{-(OH)CO}_2\text{H}$ ), fluorosulfonyl-benzoic acid ( $\text{FSO}_2\text{C}_6\text{H}_4\text{CO}_2\text{H}$ ), hydroxybenzoic acid ( $\text{HOC}_6\text{H}_4\text{CO}_2\text{H}$ ), and phthalic acid ( $\text{C}_6\text{H}_4\text{-1,2-(CO}_2\text{H)}_2$ ). Al-<sup>10</sup>though they are preferred for use with colloidal silver, with the various benzoic acid stabilizers being most preferred, these organic acid stabilizers may also be used with the silver salts identified above.

An inorganic acid is used to reduce the pH of the silver- and stabilizer-containing composition. In gen-<sup>15</sup>eral, 75% aqueous phosphoric acid, 65% aqueous nitric acid or 69% aqueous sulphuric acid is used. Hydro-bromic acid Hydrochloric acid or boric acid are like-wise suitable. It is recommended that the same acid be used as will later be used to stabilize the commercial<sup>20</sup> hydrogen peroxide, which will be mixed with the concentrate. The acid serves as a pH regulator, but also acts as an additional stabilizer for the silver compound and for the ready-to-use product. For this reason, it is neces-<sup>25</sup>sary to add acid in excess of the amount required to adjust pH. The entire amount of acid in the finished concentrate is at least equimolar to the existing amount of silver, preferably in excess, for example, of about 100 g of aqueous acid per liter of concentrate.

If the concentrate, after it is mixed with hydrogen<sup>30</sup> peroxide to become a ready-to-use disinfectant, will be exposed for a long time to UV-radiation, for example solar radiation, etc., as in the case of an open-air swim-ming pool, it is advisable to add gelatin to the concen-<sup>35</sup>trate at at least 20° C. before homogenization; in gen-eral, this addition amounts to about 20 g per liter of concentrate and protects the silver against the effects of UV-radiation.

It is advantageous to manufacture the concentrate at<sup>40</sup> a minimum temperature of 20° C. and under red light until bottling is completed, for example, in containers of colored glass or plastic without pigment.

The concentrate of the present invention is of unlim-<sup>45</sup>ited durability, even at tropical temperatures, and re-mains, in contrast to well-known products, a clear, colorless, homogenous fluid (similar to water) which does not thicken after a long period of time. This lasting homogeneity of the product is a significant advantage because it permits optional future portioning of the<sup>50</sup> concentrate without the renewed homogenizing that was necessary until the development of the present invention.

The concentrate may be mixed with hydrogen perox-<sup>55</sup>ide of 35 to 50% by weight (in water) at a volume ratio from 1:99 to 1:199. The resulting ready-to-use agent has an Ag concentration between 0.05 and 0.1% by weight. At a ratio of over 1:199, the product loses the silver/hy-<sup>60</sup>drogen peroxide synergy characteristics. At a ratio of less than 1:99, the possibility arises that the silver will precipitate from the admixture. The product within the above-mentioned limits has a shelf-life of at least 2 years.

The concentration of added hydrogen peroxide can,<sup>65</sup> for certain applications, easily amount to 35% by weight, yet the product decomposes more quickly and the rate of disinfection is lower than that obtained by using 50% by weight hydrogen peroxide.

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No renewed homogenizing of the concentrate before mixing it with hydrogen peroxide is necessary, due to the stable homogeneity of the concentrate.

Disinfectants of this type, mixed with hydrogen per-<sup>5</sup>oxide and ready to use, can then be bottled in customary transport and marketing containers, which should be equipped with a safety device to reduce any excessive gas pressure, for example a pressure relief valve. The chemical agents can last in such containers at a tempera-<sup>10</sup>ture between 4 and 25° C. for about 2 years without any danger. At higher temperatures, a longer period of storage is not recommended, since hydrogen peroxide tends to release gas.

The disinfectants invented are suitable for sterilizing<sup>15</sup> water, foodstuffs and animal feeds, stationary surfaces, etc. The concentrate is added in very small amounts, in general amounting to about 10 to 75 ppm when added as a so-called "material disinfecting agent," to such things as bath water, drinking water, foodstuffs, cooking wa-<sup>20</sup>ter, etc., or is used as a 0.1 to 2% by weight solution for surface disinfection. The products or objects disinfected with these new agents exhibit absolutely no change in smell, taste or appearance, are non-toxic, produce nei-<sup>25</sup>ther skin irritations nor other health impairments or injuries, and are completely inert with respect to ordi-nary materials such as concrete, wood, stone, glass, metals, porcelain, ceramic, plastics, textiles, etc.

The ready-to-use agent has a pH of about 2, and<sup>30</sup> should be adjusted to this pH, if necessary, preferably by adding an additional quantity of the inorganic acid already present in the composition. In contrast to chlo-rine, the agent itself does not change the pH of the product to which it is added, primarily because only<sup>35</sup> small amounts need be used. It can be used within broader temperature limits than chlorine, also, namely between 0 and 95° C., and the disinfecting effect in-creases with the rising temperature.

In the concentrations given above, the new agents are<sup>40</sup> widely able to annihilate pathogenic germs. They fight, among other things, Gram-positive and Gram-negative bacteria, bacteriophage, viruses, etc., such as *E. coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Mycobacterium tuber-*<sup>45</sup>*culosis*, *Candida albicans*, etc.

The new agents are especially suitable for steriliza-<sup>50</sup>tion: in the foodstuffs and animal feed industries, includ-ing the canning industry, for preservation of fresh prod-ucts, for processing of fish for partial or complete pre-serving, and for disinfection in slaughterhouses; in the beverage industry, and in breweries, for the preparation of mineral water, in the production of wine and spirits, in production of fruit and vegetable juices, for disinfec-<sup>55</sup>tion of bottles and casks, and in water to be added to concentrates; in water disinfection for drinking water in wells or storage containers, in swimming pools, and in hot whirlpools; as well as in dairies, in farming, in the chemical and pharmaceutical industries, in laboratories and hospitals, for fighting diseases, etc.

In the following examples, all percentages are rela-<sup>60</sup>tive to weight, provided no other notations are given.

#### EXAMPLE I

At over 20° C. and under red light, 75% phosphoric<sup>65</sup> acid was, slowly and by portions, added and stirred into 1 liter fully desalted water until the pH was equal to or less than 1.6. The mixture was stirred and heated to 55° C. and, with stirring, was mixed with 140 g silver ni-

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trate. Stirring continued until complete homogenization occurred.

The solution was left to cool to about 25 to 30° C.; 75% phosphoric acid was then slowly stirred in by portions so that the entire quantity of aqueous phosphoric acid equalled 100 g in solution. The mixture was then, without stirring, allowed to reach room temperature (20°-25° C.), at which point 50 g tartaric acid was added.

The resulting mixture was next homogenized, in this particular case after addition of 20 g of gelatin, which was stirred in at a temperature which exceeded 20° C. A crystal-clear solution resulted. The same result has been reached when, in place of aqueous phosphoric acid, 65% nitric acid or 37% Hydrochloric acid or 69% sulphuric acid is added for a total addition of 100 g acid, and/or when the silver nitrate is replaced by 135 g silver sulfate or 124 g silver chloride or 176 g sodium/silver chloride complex (AgNaCl<sub>2</sub>), and/or when, instead of tartaric acid, 50 g citric acid or 50 g of a mixture of citric acid and tartaric acid is used.

#### EXAMPLE II

To manufacture 11 liters of concentrate, 1 liter of distilled water was, slowly and by portions, mixed at about 55° C. with 850 g sodium or potassium benzoate. Stirring was then stopped, and the mixture was kept at 55° C.

In a separate container, 8350 g of colloidal silver solution (12 g per liter Ag in 5% hydrous polyhydroxyl monocarboxylic acid solution from Degussa AG) were added slowly and by portions to 75% phosphoric acid, 65% nitric acid or 69% sulphuric acid to yield a pH less than or equal to 1.6, and the mixture was warmed to 55° C.

To this mixture 1100 g of the aqueous sodium or potassium benzoate prepared above were then added, and the mixture was homogenized well. It was subsequently allowed to cool, without stirring, at room temperature (20°-25° C.). To the cooled mixture was then added, by stirring, an amount of the same inorganic acid to adjust pH such that the entire amount equalled 800 g; the addition was made slowly and by stirring in portions. This caused the mixture to homogenize well. Through addition of 200 g of gelatin at a minimum of 20° C. and renewed homogenizing, a concentrate was obtained which was well suited for disinfecting swimming pools; ultraviolet radiation did not affect the composition during use.

#### EXAMPLE III

Five liters of concentrate, obtained according to Example I, were mixed at volume ratio 1:99 with 50% by volume H<sub>2</sub>O<sub>2</sub>, at room temperature, under red light, and in a retort made of stainless steel, until homogenized. After formation of bubbles ceased, the product was bottled. This product was especially suitable for long-term disinfection, particularly at high temperatures, such as an additive for hot whirlpools, recycled water for water-cleaning installations, etc., preferably in a concentration from 40 to 75 ppm.

#### EXAMPLE IV

Eleven liters of concentrate, obtained according to Example II, were processed at a ratio of 1:19.9, as in Example III, with 50% by volume H<sub>2</sub>O<sub>2</sub> into a finished product. The resulting product is suitable, for example, for disinfection of drinking water in a concentration

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from 10 to 34 ppm, for the surface disinfection in a 0.1 to 2% solution.

Although the invention has been described with reference to specific methods and materials, the invention is to be limited only insofar as is set forth in the accompanying claims.

I claim:

1. A process for preparing a storage stable, clear concentrate of unlimited durability which, upon admixture with hydrogen peroxide, forms a disinfectant, comprising:

(a) admixing an inorganic acid with water, said water being selected from the group consisting of distilled water and fully desalted water, to yield aqueous inorganic acid having a pH less than or equal to 1.6;

(b) admixing said aqueous acid at a temperature between 50 and 60° C. with a silver composition, selected from the group consisting of silver salts and silver salt complexes, in an amount which yields 95-105 g Ag per liter of the final concentrate;

(c) cooling the resultant admixture to between 25 and 30° C., and adding such an amount of the same acid as was used for adjusting the pH of the water whereby the total amount of said acid is at least equimolar with the amount of silver present;

(d) adding to said admixture an organic acid stabilizer, and optional gelatin, at 20 to 25° C.; and

(e) homogenizing the obtained mixture.

2. The process according to claim 1 wherein the inorganic acid is selected from the group consisting of phosphoric acid, nitric acid and sulfuric acid, preferably in an amount of 100 g per liter water.

3. The process according to claim 1 wherein the silver compound added in step (b) is selected from the group consisting of silver nitrate, silver sulfate, silver chloride, sodium/silver chloride complex, silver benzoate, silver carbonate, silver fluoride, silver oxide and silver oxide, in an amount which yields approximately 95-105 g Ag per liter of the final concentrate; and the organic acid added in step (d) is selected from the group consisting of tartaric acid, citric acid and compositions containing both tartaric acid and citric acid, preferably in an amount of 100 g per liter water.

4. The process for the manufacture of a disinfectant, comprising admixing, at a temperature of at least 20° C. and optionally under red light, a concentrate, prepared in accordance with the process of one of claims 1, 2, or 3, with 35-50% by volume aqueous hydrogen peroxide at a ratio between 1:99 and 1:199 concentrate to aqueous hydrogen peroxide, whereby the ratio is chosen to yield a resultant composition having an Ag concentration between 0.05 and 0.1% by weight.

5. A process for preparing a storage stable, clear concentrate of unlimited durability which, upon admixture with hydrogen peroxide, forms a disinfectant, comprising:

(a) admixing an inorganic acid with an aqueous solution containing colloidal silver solution in 5% aqueous polyhydroxymonocarboxylic acid, to yield a solution having a pH less than or equal to 1.6;

(b) heating said aqueous solution to between 50 and 60° C. and adding thereto a solution of an organic stabilizer in distilled or fully desalted water having about the same temperature in an amount which



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yields approximately 9.5–10.5 g Ag per liter of the final concentrate;

(c) cooling the admixture to 20° to 25° C.;

(d) adding thereto such an amount of the same inorganic acid as was used for adjusting the pH that the total amount of inorganic acid is at least equimolar with the amount of silver present;

(e) optionally adding gelatin; and

(f) homogenizing the admixture.

6. The process according to claim 5 wherein the colloidal silver is added in the form of an aqueous solution which further contains about 5% by weight of polyhydroxyl carboxylic acid, and wherein a benzoic acid stabilizer, and optional gelatin, are added.

7. The process according to any of claims 1, 5, 2, 3 or 6 wherein said inorganic acid is mixed with said water at a temperature of at least 20° C.

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8. The product prepared in accordance with the process of any of claims 1, 5, 2, 3, or 6.

9. The process according to claim 5 wherein the inorganic acid is selected from the group consisting of phosphoric acid, nitric acid and sulfuric acid, preferably in an amount of 100 g per liter water.

10. The product prepared in accordance with the process of claim 7.

11. The process for the manufacture of a disinfectant, comprising admixing, at a temperature of at least 20° C. and optionally under red light, a concentrate, prepared in accordance with the process of one of claims 5, 6 or 7, with 35–50% by volume aqueous hydrogen peroxide at a ratio between 1:9.9 and 1:19.9 concentrate to aqueous hydrogen peroxide, whereby the ratio is chosen to yield a resultant composition having an Ag concentration between 0.05 and 0.1% by weight.

12. The disinfectant product obtained in accordance with the process of either of claims 4 or 11.

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D2

<p>95-261152/34 A97 D22 E32 HAYA/ 93.12.10 HAYASHIK *JP 07165510-A 93.12.10 93JP-355477 (95.06.27) A01N 43/16, 25/02, 59/16, 37/04 Disinfectant soln. - comprises aq. soln. contg. inorganic acid and/or organic acid, chitosan and silver cpd(s). C95-118737 Addnl. Data: MORIZAKI S (MORI/)</p>	<p>A(12-V3C1) D(9-A1C) E(35-B)</p>
<p>Disinfectant solns. or their condensates comprises aq. soln. contg. inorganic acid and/or organic acid, chitosan, and silver cpds. (pref. silver nitrate-contg. aq. soln.) in 0.5-100µ g/ml, pref. 5-50 µ/ml of the final concn. Also claimed are disinfectant solns., or their condensates contg. colloidal particles of activated silver prepd. by irradiating the light to the disinfectant solns. or their condensates.</p> <p><u>USE</u> The disinfectant solns. or their condensates are useful for sterilisation of hands, fingers, mats, sheets or walls in the hospitals.</p> <p><u>ADVANTAGE</u> The disinfectant solns. have wide antibacterial spectrum to bacterials such as P. aeruginosa, E. coli, or MRSA 15, and good</p>	<p>storage stability (e.g. 2 years at room temp.).</p> <p><u>PREFERRED COMPONENTS</u> Inorganic acids (e.g. hydrochloric acid, or phosphoric acid), and/or organic acids (e.g. citric acid or acetic acid) in 0.1-10% of the concn. are used. Chitosan in 10-95% of deacetylation degree is used in 0.1-5%, pref. 0.5-2%. The silver colloids have &lt;10µ pref. 0.5µ prepd. by irradiating the light of 50,000 Lux/hour.</p> <p><u>EXAMPLE</u> Chitosan (10g) was added to a mixt. of water (900 ml) and citric acid (10g), and stirred overnight. A soln. of silver nitrate (500 mg), and water (100 ml) was dropped into the mixt., and stirred with irradiation of fluorescent lamp a day to give a disinfectant soln. contg. activated silver of &lt;0.5µ. (10pp62DwgNo.0/2)</p> <p>JP 07165510-A</p>

[*Jour. Indian Chem. Soc.*, Vol. 84, No. 9, 1957]

D3

## ON THE COMPOSITION OF SILVER CITRATE

By KRISHNA C. MATHUR AND ARUN K. DEY

In previous communications (Dey, *Compt. rend. Acad. Sci., U.S.S.R.*, 1947, 83, 1047; *J. Coll. Sci.*, 1948, 3, 473) the formation of a soluble complex between silver and citrate ions has been described. When a soluble citrate is added to a silver nitrate solution, a white curdy precipitate is obtained, which dissolves in excess of citrate, forming the argentocitrate complex (Mukherji and Dey, *Anal. Chim. Acta*, 1955, 12, 501; *Z. anal. Chem.*, 1955, 146, 94). The composition of the precipitated silver citrate seems to have remained uninvestigated and it is considered worthwhile to investigate its composition. A similar precipitate is also formed by adding sodium, potassium or ammonium hydroxide solution to the colorless solution obtained by dissolving silver citrate in an excess of citric acid solution. In this note we have found the composition of the white curdy precipitate to be  $\text{Ag}_3\text{Cit}$  in every case.

**Procedure.**—Standard solutions of silver nitrate, sodium citrate, citric acid and sodium, ammonium and potassium hydroxides were prepared using BDH-Analar reagents and dissolving in double-distilled water. All the solutions were standardised by the usual methods.

In the first set of experiments 25 c.c. of 0.125 M silver nitrate was taken and 1 M solution of sodium dihydrogen citrate was added dropwise till precipitation was complete. The precipitate was kept overnight, filtered in a sintered glass crucible and was thoroughly washed with water. The precipitate was dried in an air-oven at 100-110°, and then analysed for its silver content which was found to be 62.39% (Ag calc. for  $\text{Ag}_3\text{Cit}$ : 63.13%).

In a second set of experiments equal volumes of 0.125 M silver nitrate and 1 M citric acid were mixed and to the mixture varying amounts of 2 M sodium, potassium or ammonium hydroxide were added. The precipitates obtained were washed and analysed after drying. In every case, the composition was found to be  $\text{Ag}_3\text{Cit}$ .

It is thus concluded that the precipitate in all cases is  $\text{Ag}_3\text{Cit}$ , i.e., silver replaces carboxylic hydrogen in citric acid to produce the insoluble citrate, and that the hydroxylic hydrogens are not involved in the formation of precipitated silver citrate. It is also evident that the soluble argentocitrate is unstable in presence of alkali and is decomposed to silver citrate on the addition of hydroxyl ions. Further work on complex citrates is in progress.

The authors are thankful to the Scientific Research Committee, U. P. Govt., for supporting the work and for the award of a research assistantship to one of them (K.C.M.).

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Received July 15, 1957.

**United States Patent** [19]

Matsuo et al.

[11] Patent Number: **4,755,268**[45] Date of Patent: **Jul. 5, 1988**[54] **PROCESS AND APPARATUS FOR PRODUCING SILVER-IONIC WATER**

[76] Inventors: Yoshiaki Matsuo, 2-19-11, Omorihoncho, Ota-ku, Tokyo; Jin-ichi Ito, 1-2-1, Nishiwaseda, Shinjuku-ku, Tokyo; Katsue Oshima, 4-26-9, Shichirigamahigashi, Kamadura-shi, Kanagawa-ken, all of Japan

[21] Appl. No.: 50,437

[22] Filed: May 18, 1987

## [30] Foreign Application Priority Data

May 28, 1986 [JP] Japan ..... 61-123133  
Dec. 26, 1986 [JP] Japan ..... 61-314792

[51] Int. Cl.<sup>4</sup> ..... C02F 1/46

[52] U.S. Cl. .... 204/149; 204/131; 204/130; 204/269

[58] Field of Search ..... 204/130, 131, 149, 86, 204/269

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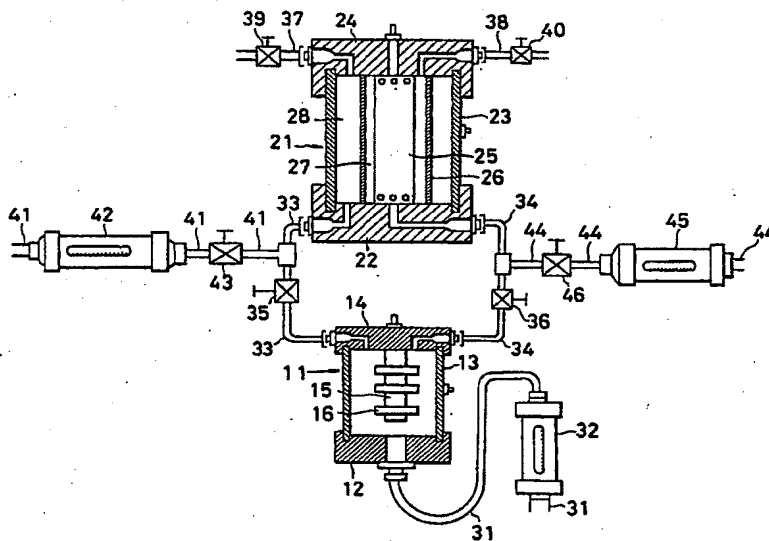
Primary Examiner—R. L. Andrews  
Attorney, Agent, or Firm—Beveridge, DeGrandi & Weilacher

[57] **ABSTRACT**

A process for producing silver-ionic water, comprising: a step of passing water through a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode, under the application of a voltage, to dissolve out silver ions; and a step of passing the water in which said silver ions have been dissolved out, through either one or both of an anode compartment and a cathode compartment of a second electrolytic cell chamber containing an anode and a cathode and separated into said anode compartment and said cathode compartment with a diaphragm formed between both of said electrodes.

The process is useful for producing acidic silver-ionic water and/or alkaline silver-ionic water without causing the formation of colloids even under relatively higher silver ion concentration.

Also disclosed are useful apparatus for practicing the above processes.

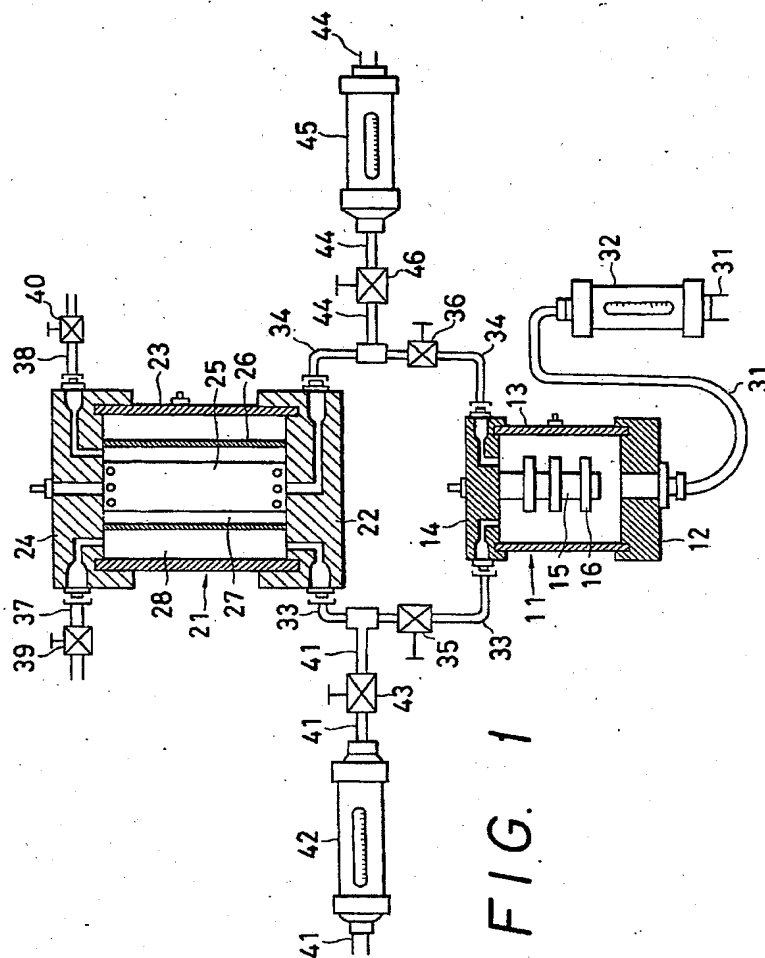
**10 Claims, 3 Drawing Sheets**

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FIG. 2

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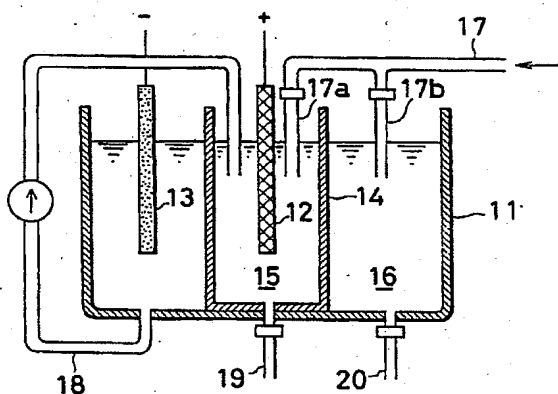


FIG. 3  
(PRIOR ART)



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## PROCESS AND APPARATUS FOR PRODUCING SILVER-IONIC WATER

### BACKGROUND OF THE INVENTION

This invention relates to a process for producing silver-ionic water (or silver ion-containing water) used in the field of, for example, sanitary administration in processing, distribution and selling of foods, soil conditioning and cultivation promotion in agriculture, environmental sanitation in medical care, etc., and an apparatus for producing the same.

Silver-ionic water, which is less dangerous for human bodies and has a bactericidal action, has recently attracted notices as potable water, sterilized water and so forth.

In the present invention, the actions expected in silver ions include a bacteriostatic action against bacteria, an action of supplying active oxygen to roots of plants, an action of supplying OH ions for the purpose of promoting photosynthesis in the cells of plants with a lower amount of light, and actions such as elimination of pains, hemostasis and promotion of a vulnerary action. Of these, the bacteriostatic action originates from the oxidation catalytic function inherent in silver acting on a cell membrane of bacteria, the silver concentration, and the pH of ionic water. Upon contact of silver ions with a bacterial body, the catalytic function of silver may cause a radical reaction in the unsaturated aliphatic acid constituting a hydrophobic part of a cell membrane of bacteria to hydrolyze the same. The cell membrane of bacteria thereby loses its function, aiming at stopping vital activities by inflow of water, and DNA and constituents of a nuclear membrane may not be affected by the catalytic function of silver to remain in a sound form. Moreover, silver tends to be little accumulated inside a living body as compared with other metallic elements. From these facts, silver ions are considered to be little dangerous for human bodies and, from a genetic viewpoint also, cause no disorder in a fetus that may affect a next generation, and therefore, if conditions are adjusted, has a possibility to serve as a new bacteriostatic agent substituting conventional chemical medications.

Conventionally, the silver-ionic water has been produced by an apparatus embodying a process called a Katadyn process. This apparatus comprises an anode and a cathode, wherein the anode is provided with silver, and water is allowed to pass while a voltage is applied between both electrodes, whereby silver is ionized and dissolved out to give silver-ionic water.

However, the apparatus mentioned above is involved in a problem that the resulting silver-ionic water is substantially neutral, and, under the neutral condition, silver ions may be adsorbed on proteins and can have only a little chance for direct contact with the inside of a cell of a bacterial body when proteins are contained in water or bacteria are in contact with proteins, resulting in an extreme lowering of its bacteriostatic action. Specifically speaking based on an experimental work made by the present inventors, this silver-ionic water was brought into contact with bacteria in an aqueous solution in which proteins are dissolved, and also, separately, with bacteria adhered to proteins, but, because of the neutral environment, metallic ions were vigorously bonded to proteins, releasing ions, and was not able to enter the cells of bacteria when brought into contact

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with the bacteria, with the loss of the effect by silver ions and no achievement of the object.

To cope with the problem, the present inventors have made intensive studies. As a result, they have found that silver-ionic water having a given silver ion concentration under the acidic condition of pH 5 or less or the alkaline condition of pH 8 or more may have a remarkable bacteriostatic action against bacteria present together with proteins, with a silver ion concentration that may be less dangerous for human bodies, and that the acidic silver-ionic water has an action of supplying O<sub>2</sub> to roots of plants and the alkaline silver-ionic water, when supplied to plant cells, has an action of promoting photosynthesis.

As an apparatus for producing acidic or alkaline silver-ionic water, known is an apparatus as disclosed in Japanese Unexamined Patent Publication No. 97088/1985, for example. As shown in FIG. 3, this apparatus comprises an electrolytic cell 11 provided with an anode 12 comprising a silver electrode, and a cathode 13 comprising a carbon electrode or the like, wherein a cylindrical ion-exchange diaphragm 14 is disposed around the anode 12, which diaphragm 14 defines an anode compartment 15 and a cathode compartment 16 which are separated from each other. Water passes through branch pipes 17a and 17b of a conduit 17, and is led into the anode compartment 15 and the cathode compartment 16, respectively. One end of a feedback pipe 18 is connected to the bottom of the cathode compartment 16, and the other end of the feedback pipe 18 opens to an upper portion of the anode compartment 15. Water in the cathode compartment 15 circulates to the anode compartment 15 through the feedback pipe 18. Also, a take-out pipe 19 is connected to the bottom of the anode compartment 15 and another take-out pipe 20 is connected to the bottom of the cathode compartment 16.

Thus, in this prior art apparatus, water is introduced into the anode compartment 15 and the cathode compartment 16 through the branch pipes 17a and 17b of the conduit 17, and allowed to circulate from the cathode compartment 16 to the anode compartment 15 through the feedback pipe 18. Further, the water in the anode compartment 15 is taken out from the take-out pipe 19 and the water in the cathode compartment 16 is taken out from the take-out pipe 20. By applying a direct-current voltage to the anode 12 and the cathode 13, silver on the anode 12 is ionized to dissolve. Acidic silver-ionic water is taken out from the take-out pipe 19 of the anode compartment 15 and alkaline silver-ionic water is taken out from the take-out pipe 20 of the cathode compartment 16. Here, since the water in the cathode compartment 16 is allowed to circulate to the anode compartment 15 through the feedback pipe 18, the water taken out from the anode compartment 16 and the cathode compartment 16 may have substantially the same silver ion concentration.

However, in the above apparatus, a high voltage have had to be applied in order to attain the desired EC value by applying a voltage to the anode 12 and the cathode 13. Therefore, because of the silver electrode provided on the anode 12, if it is attempted to increase the silver ion concentration of the water to be taken out, silver particles beings to precipitate to form colloids when the silver ion concentration reaches a certain level. Thus, there has been a limit in increasing the silver ion concentration. Moreover, there has been a fear of adversely affecting human bodies when a large

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quantity of silver grains are precipitated to form colloids.

### SUMMARY OF THE INVENTION

An object of the present invention is to provide a process, and an apparatus, for producing silver-ionic water, that can produce acidic silver-ionic water or alkaline silver-ionic water without causing the formation of colloids even under relatively higher silver ion concentration, and also that can produce silver-ionic water having electric conductance of 350 to 2000  $\mu\text{S}/\text{cm}^3$  at which the acidic side electrochemical potential can be increased.

Another object of the present invention is to provide a process, and an apparatus, for producing long term stable silver-ionic water, which makes it possible to produce silver-ionic water that can stably maintain a uniform silver ion concentration over a long period of time and also can attain high operational effects against target matters such as bacteria under relatively low concentration and even in the presence of proteins mixed.

According to a first embodiment of the present invention there is provided a process for producing silver-ionic water, comprising;

a step of passing water through a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode, under the application of a voltage, to dissolve out silver ions; and

a step of passing the water in which said silver ions have been dissolved out, through either one or both of an anode compartment and a cathode compartment of a second electrolytic cell chamber containing an anode and a cathode and separated into said anode compartment and said cathode compartment with a diaphragm formed between both of said electrodes.

As an apparatus for carrying out the above process according to the first embodiment of the present invention, there is provided an apparatus for producing silver-ionic water, comprising;

a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode, a second electrolytic cell chamber containing an anode and a cathode and separated into an anode compartment and a cathode compartment with a diaphragm formed between both of said electrodes, and

at least one of a flow path in which water is passed from said first electrolytic cell chamber through the anode compartment of said second electrolytic cell chamber and flowed out, and a flow path in which water is passed from said first electrolytic cell chamber through the cathode compartment of said second electrolytic cell chamber and flowed out.

According to a second embodiment of the present invention, there is provided a process for producing silver-ionic water, comprising;

(a) subjecting water to a demineralizing treatment to decrease ionic substances in the water;

(b) adding an organic carboxylic acid to the ion-decreased water;

(c) passing the water to which the organic carboxylic acid has been added, through a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode, under the application of a voltage, to dissolve out silver ions;

(d) passing under the application of a direct-current voltage the water in which said silver ions have been dissolved out, through at least a cathode compartment

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side of a second electrolytic cell chamber containing an anode and a cathode and separated into an anode compartment and the cathode compartment with a diaphragm formed between both of said electrodes; and

(e) adding sodium chloride to the water having been passed through said cathode compartment so that silver may be present in the water as a soluble complex.

As an apparatus for carrying out the above process according to the second embodiment of the present invention, there is provided an apparatus for producing silver-ionic water, comprising;

an ion exchange chamber filled with an iron exchange material;

a first mixing bath for adding an organic carboxylic acid;

a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode;

a second electrolytic cell chamber having an anode and a cathode and separated into an anode compartment and a cathode compartment with a diaphragm formed between both of said electrodes;

a second mixing bath for adding sodium chloride; and a flow path in which water is flowed out after being passed from said ion exchange chamber through said first mixing bath, said first electrolytic cell chamber, the cathode compartment of said second electrolytic cell chamber and said second mixing bath.

### BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a partially cross-sectional view showing an embodiment of the apparatus for producing silver-ionic water according to the first embodiment of the present invention;

FIG. 2 is a partially cross-sectional view showing another embodiment of the apparatus for producing silver-ionic water according to the second embodiment of the present invention; and

FIG. 3 is a cross-sectional view showing a conventional apparatus for producing silver-ionic water according to a prior art.

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

According to the present invention, acidic silver-ionic water such as  $\text{Ag}^+$ -containing water can be obtained by dissolving out silver ions in water in the first electrolytic cell chamber and passing this water through the anode compartment of the second electrolytic cell chamber, and alkaline silver-ionic water such as water containing  $\text{Ag}(\text{OH})_2^-$ ,  $\text{Ag}_2(\text{OH})^+$ , etc. can be obtained by passing the water treated in the first electrolytic cell chamber through the cathode compartment of the second electrolytic cell chamber. Also, in the apparatus for carrying out this process, the voltage, current, and flow rate of water in the first electrolytic cell chamber may be controlled to attain a desired silver ion concentration, without formation of colloids of silver particles even when the silver ion concentration has been increased. This is because the desired silver ion concentration is controlled in the first electrolytic cell chamber and the water having been made silver-ionic is made acid or alkaline in the second electrolytic cell chamber. According to the apparatus of the present invention, it is also possible to produce an acidic silver-ionic water having an increased acidic side electrochemical potential and an alkaline silver-ionic water to have respectively the desired pH value, EC value and amount of dissolved oxygen by controlling the voltage,

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current, and flow rate of water in the second electrolytic cell chamber.

According to a preferred embodiment of the present invention, there are provided, in addition to the above water flow paths, a flow path in which the water is directly passed through the above anode compartment of the second electrolytic cell chamber and flowed out, and another flow path in which the water is directly passed through the above cathode compartment of the second electrolytic cell chamber and flowed out. According to this embodiment, water is passed from the first electrolytic cell chamber to the second electrolytic cell chamber and at the same time another water is directly passed through the cathode compartment of the second electrolytic cell chamber, so that, while controlling the flow rate or both the water, desired acidic silver-ionic water only can be taken out from the anode compartment of the second electrolytic cell chamber. Alternatively, water is passed from the first electrolytic cell chamber to the anode compartment of the second electrolytic cell chamber and at the same time another water is directly passed through the anode compartment of the second electrolytic cell chamber, so that, while controlling the flow rate of both the water, desired alkaline silver-ionic water only can be taken out from the cathode compartment of the second electrolytic cell chamber.

FIG. 1 illustrates an example of the apparatus for producing silver-ionic water according to the first embodiment of the present invention.

The apparatus of FIG. 1 chiefly comprises a first electrolytic cell chamber 11 and a second electrolytic cell chamber 21. The first electrolytic cell chamber 11 is enclosed by a bottom plate 12 comprising a non-conductive material, a cathode plate 13 surrounding the outer periphery and made of stainless steel or the like, and a cover plate 14 made of a non-conductive material. Through the cover plate 14, an anode rod 15 is inserted in the manner that the lower end portion thereof may be inward extended. Also, the anode rod 15 is provided with silver or silver alloy 16.

To the bottom plate 12, a first water-feeding pipe 31 is connected via a flowmeter 32 provided on the way of the pipe so that water may be introduced into the first electrolytic cell chamber 11 through the pipe 31. To the cover plate 14, connecting pipes 33 and 34 are connected so that the water in the first electrolytic cell chamber 11 can be led out of it. The connecting pipe 33 is provided with a valve 35 and the connecting pipe 34 is provided with a valve 36.

On the other hand, the second electrolytic cell chamber 21 is enclosed by a bottom plate 22 made of a non-conductive material, a cathode plate 23 surrounding the outer periphery and made of stainless steel or the like, and a cover plate 24 made of a non-conductive material. To the cover plate 24, an anode plate 25 is fixed in the manner that it may be inward extended. In the inside of the second electrolytic cell chamber 21, a cylindrical diaphragm 26 is arranged so as to surround the anode plate 25, which diaphragm 26 partitions an anode compartment 27 and a cathode compartment 28. The diaphragm 26 has properties that it allows ions such as  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ , etc. to pass from the anode compartment 27 to the cathode compartment 28 and allows ions such as  $\text{Cl}^{-}$ ,  $\text{SO}_4^{--}$ ,  $\text{HCO}_3^{-}$ , etc. to pass from the cathode compartment 28 to the anode compartment 27, to prevent reverse flowing of them.

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The aforesaid connecting pipes 33 and 34 are connected to the bottom plate 22, of which the connecting pipe 33 is led to the above cathode compartment 28 and the connecting pipe 34 is led to the above anode compartment 27. Lead-out pipes 37 and 38 are connected to the cover plate 24, of which the lead-out pipe 37 is led to the cathode compartment 28 and the lead-out pipe 38 is led to the anode compartment 27. The lead-out pipes 37 and 38 are provided with valves 39 and 40, respectively.

A second water-feeding pipe 41 is connected between the valve 35 of the connecting pipe 34 and the bottom plate 22 via a flowmeter 42 and a valve 43. A third water-feeding pipe 44 is connected between the valve 36 of the connecting pipe 34 and the bottom plate 22 via a flowmeter 44 and a valve 46.

To simultaneously obtain the acidic silver-ionic water and the alkaline silver-ionic water by use of this apparatus, valves 36, 39 and 40 are opened, and valves 43 and 46 are closed, so that water is fed from the first water-feeding pipe 31. The water thus fed from the first water-feeding pipe 31 is led into the first electrolytic cell chamber 11, where silver ions are formed. Then, part of the water containing silver ions is led into the anode compartment 27 of the second electrolytic cell chamber 21 through the connecting pipe 34, and taken out from the lead-out pipe 38 in the form of acidic silver-ionic (e.g.  $\text{Ag}^{+}$ ) water formed in the anode compartment 27 and having an electric conductance of 350 to 2000  $\mu\text{S}/\text{cm}^3$ . At the same time therewith, remaining part of the water containing silver ions is led into the cathode compartment 28 of the second electrolytic cell chamber 21 through the connecting pipe 33, and taken out from the lead-out pipe 37 in the form of alkaline silver-ionic (e.g.  $\text{Ag}(\text{OH})_2^{-}$ ,  $\text{Ag}_2(\text{OH})^{+}$ ) water formed in the cathode compartment 28.

To obtain only the acidic silver-ionic water by use of the above apparatus, procedures may be taken as follows: Valves 36, 39, 40 and 43 are opened, and valves 35 and 46 are closed. Under such a state, water is fed under pressure from the first water-feeding pipe 31 and the second water-feeding pipe 41, respectively. The water fed from the first water-feeding pipe 31 passes through the first electrolytic cell chamber 11, the connecting pipe 34 and the anode compartment 27 of the second electrolytic cell chamber 21, and flows out from the lead-out pipe 38. Also, the water fed from the second water-feeding pipe 41 passes through the connecting pipe 33 and the cathode compartment 28 of the second electrolytic cell chamber 21, and flows out from the lead-out pipe 37. Here, the flow rate of the water flowing through both the flow paths is controlled as necessary while watching the flowmeters 32 and 42. Under such a state, a direct-current voltage is applied between the anode rod 15 and cathode plate 13 of the first electrolytic cell chamber 11, and also a direct-current voltage is applied between the anode plate 25 and cathode plate 23 of the second electrolytic cell chamber 21. Taking these procedures, the water fed from the first water-feeding pipe 31 is led into the first electrolytic cell chamber 11, where silver ions are formed, led into the anode compartment 27 of the second electrolytic cell chamber 21 through the connecting pipe 34, and taken out from the lead-out pipe 38 in the form of acidic silver-ionic water such as  $\text{Ag}^{+}$  ionic water formed in the anode compartment 27 and having an electric conductance of 350 to 2000  $\mu\text{S}/\text{cm}^3$ .

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To obtain only the alkaline silver-ionic water by use of the above apparatus, valves 35, 39, 40 and 46 are open, and valves 36 and 43 are closed. Water is fed from the first water-feeding pipe 31 and the third water-feeding pipe 44, respectively. Taking these procedures, the water fed from the first water-feeding pipe 31 is led to the first electrolytic cell chamber 11, where silver ions are formed, led into the cathode compartment 28 of the second electrolytic cell chamber 21 through the connecting pipe 33, and taken out from the lead-out pipe 39 in the form of alkaline silver-ionic water such as  $\text{Ag}(\text{OH})_2^-$  or  $\text{Ag}_2(\text{OH})^+$  water formed in the cathode compartment 28.

In this apparatus, acidic or alkaline silver-ionic water having a desired silver ion concentration and pH can be obtained by controlling the flow rate of the water flowing through the respective flow paths, the voltage to be applied to the anode rod 15 and cathode plate 13 of the first electrolytic cell chamber 11 and the voltage to be applied to the anode plate 25 and cathode plate 23 of the second electrolytic cell chamber 21.

In this instance, the controlling of the silver ion concentration can be varied chiefly depending on the voltage applied to the anode rod 15 and the cathode plate 13 of the first electrolytic cell chamber 11. Speaking more precisely, the silver ion concentration can be controlled according to the product of the applied voltage in the first electrolytic cell chamber 11 and the electric current flowing in water at that time; in other words, the electric power consumption. Since the electric current flowing in water can be determined according to the applied voltage, the electric conductance of water and the flow rate of water, it anyway follows that the silver ion concentration can be controlled according to the applied voltage, the electric conductance of water and the flow rate of water. Accordingly, it follows that the higher the applied voltage, the electric conductance of water and the flow rate of water are, the higher the silver ion concentration is made.

Results obtained by experiments actually carried out by use of the above apparatus are as follows: The flow rate of the water flowing through the first electrolytic cell chamber 11 was made to be 4.5 lit/min. The electric conductance of the water used was  $182 \mu\text{S}/\text{cm}^3$ . The voltage applied to the second electrolytic cell chamber 21 was made constant at 100V. Under these conditions, with varied voltage applied to the first electrolytic cell chamber 11, the water mentioned above was passed through the first electrolytic cell chamber 11, and the water passed there was led through two divided courses to be taken out after being passed respectively through the anode compartment 27 and cathode compartment 28 of the second electrolytic cell chamber 21. The concentration of silver ions contained in the water thus taken out was measured by atomic absorption. When the voltage applied to the first electrolytic cell chamber 11 was same, the water taken out from the anode compartment 27 and the water taken out from the cathode compartment 28 had substantially the same silver ion concentration. The relationship between the electric power consumption in the first electrolytic cell chamber 11 and the silver ion concentration of the water taken out from the second electrolytic cell chamber 21, thus obtained, is shown in Table 1. It is seen from Table 1 that the higher the electric power consumption in the first electrolytic cell chamber is, the higher the silver ion concentration is.

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For comparison, similar experiment was carried out in the following manner by use of the apparatus shown in FIG. 3: In the apparatus shown in FIG. 3, water was fed from the water-feeding pipe 17 at a rate of 4.5 lit/min, and the water divided in substantially equal amounts was led into the anode compartment 15 and the cathode compartment 16. Then, the water in the cathode compartment 16 was circulated to the anode compartment 15 through the feedback pipe 18. Further, water was taken out from the take-out pipe 19 of the anode compartment 15 and the take-out pipe 20 of the cathode compartment 16, respectively. The electric conductance of the water was  $182 \mu\text{S}/\text{cm}^3$ , same as the above. Under these conditions, the experiment was carried out with varied voltage applied to the anode 12 and the cathode 13, and the concentration of silver ions contained in the water taken out was measured by atomic absorption. In this apparatus also, the water taken out from the anode compartment 15 and the water taken out from the cathode compartment 16 had substantially the same silver ion concentration, when the voltage was kept constant. The relationship between the electric power consumption in the electrolytic cell and the concentration of silver ions contained in the water taken out, thus obtained, is shown in Table 2. It is seen from Table 2 that the silver ion concentration reaches a maximum at the electric power of 36W, and the silver ion concentration is lowered on the contrary even if the electric power is made higher. It was also actually confirmed that silver particles were precipitated to form colloids when the electric power consumption exceeded 36W.

TABLE 1

Electric power consumption (W)	Silver ion concentration (ppm)
3.4	0.70
12.6	1.50
27.9	2.14
48.4	2.94

TABLE 2

Electric power consumption (W)	Silver ion concentration (ppm)
2.6	0.55
9.4	1.15
20.7	1.58
36.0	1.94
55.0	1.79
67.7	1.70

On the other hand, in the apparatus of the present invention, the pH can be chiefly controlled by the electric power consumption in the second electrolytic cell chamber 21. This electric power consumption can be determined by the voltage applied to the anode plate 25 and the cathode plate 23, and the electric current flowing in water. The electric current flowing in water can be varied depending on the flow rate of water and the electric conductance of water, when the applied voltage is kept constant. Now, the voltage applied to the second electrolytic cell chamber 21 was made constant at 100 V, with varied flow rate of the water flowing in the anode compartment 27 and the cathode compartment 28, and the pH of the acidic silver-ionic water taken out from the anode compartment 27 and the pH of the alkaline silver-ionic water taken out from the cathode compartment 28 were respectively measured. Re-

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sults obtained are shown in Table 3 (the acidic silver-ionic water) and Table 4 (the alkaline silver-ionic water). Table 3 and Table 4 tell that the smaller the flow rate is, the more the electric current is, and, as a result, the higher the electric power consumption is. It is seen from Table 3 that the pH decreases with increase in the electric power consumption, in respect of the acidic silver-ionic water taken out from the anode compartment 27. It is also seen from Table 4 that the pH increases with increase in the electric power consumption, in respect of the alkaline silver-ionic water taken out from the cathode compartment 28.

TABLE 3

(Acidic silver-ionic water)			
Flow rate (lit/min)	Voltage (V)	Current (A)	pH value
10	100	4	5.13
9	100	5	4.25
2	100	12	3.29

TABLE 4

(Alkaline silver-ionic water)			
Flow rate (lit/min)	Voltage (V)	Current (A)	pH value
10	100	4	7.90
9	100	5	9.09
4.5	100	12	10.5

As a further feature in the apparatus of the present invention, the silver ions, having been dissolved out in the first electrolytic cell chamber 11, may be passed through the anode compartment 27 of the second electrolytic cell chamber 21, whereby the acidic silver-ionic water having a desired electrical conductance can be obtained. More specifically, the electric power consumption in the first electrolytic cell chamber 11 and the electric power consumption in the second electrolytic cell chamber 21 may be controlled, whereby the electric conductance of the acidic silver-ionic water taken out from the anode compartment 27 of the second electrolytic cell chamber 21 can be varied in the range of 350 to 2000  $\mu\text{S}/\text{cm}^3$ , and thus the electric conductance can be selected depending on what the silver-ionic water is used for. Increasing the electric conductance like this, it is made possible to improve the cell permeability of silver ion to enhance the bacteriostatic action thereof, to give electrochemical potential to trace elements to enhance a complex formation ability or an ability for metal complexes to be absorbed into plants, and further to supply active oxygen to the roots of plants.

## TEST EXAMPLE 1

Using the above apparatus according to the first embodiment of the present invention, acidic silver-ionic water each having the pH of 3.5 and a silver ion concen-

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tration of 800 ppb, 1,200 ppb, 1,600 ppb and 2,000 ppb was prepared. Thereafter, a platinum loop each of *Escherichia coli* and *Staphylococcus* was inoculated into each solution, followed by addition of a 0.1% broth, and the movements (viable cell counts) of the bacteria were observed with time lapse while culturing them at 35° C. As a result, the viable cell counts on *Escherichia coli* were as shown in Table 5.

As to *Staphylococcus*, the state of growth thereof was observed based on the turbidity of the solutions to obtain the results as shown in Table 6. Thus, the acidic silver-ionic water was found to have a remarkable bacteriostatic action.

The *Escherichia coli* and *Staphylococcus* used in the above were further cultured to repeat experiments similar to the above several times, but there appeared no bacteria resistant to silver ions, showing the same bacteriostatic action as in the first instance. Thus, there is a feature that the appearance of resistant bacteria, which has been very usual in using conventional medicaments, is not seen at all in using this silver ions.

TABLE 5

Contact time	Silver ion concentration				
	Control 0	800 ppb	1200 ppb	1600 ppb	2000 ppb
10 min	2000	160	290	60	180
2 hrs	3500	70	70	30	30
4 hrs	20000	30	20	0	0
6 hrs	55000	20	10	0	0
24 hrs	19000000	30	0	0	0
48 hrs	80000000	500000	500000	500000	0

TABLE 6

Contact time	Tests on <i>Staphylococcus</i> suspension				
	Control	800	1200	1600	2000 ppb
24 hrs	+	-	-	-	-
48 hrs	+	+	-	-	-
72 hrs	+	+	-	-	-

Next, prepared was silver-ionic water each having a silver ion concentration of 1 ppm, 0.5 ppm and 0.2 ppm, the pH of which was varied respectively to be 10, 9, 8, 7, 6, 5, 4 and 3. On the other hand, *Staphylococcus* and *Escherichia coli* were subcultured for 3 days with use of an ordinary broth medium. Also, general various bacteria were cultured from white coffee. The above *Staphylococcus*, *Escherichia coli* and general various bacteria were collected by the number of  $10^5$  for each, and brought into contact with each of the above silver-ionic water for 5 minutes to measure the viable cell counts. Results obtained were as shown in Table 7. As will be seen from Table 7, the bacteriostatic action tends to become remarkable at the pH of 5 or less or the pH of 8 or more.

TABLE 7

	<i>Staphylococcus</i>			<i>Escherichia coli</i>			General various bacteria		
	Ag ion concentration:			Ag ion concentration:			Ag ion concentration:		
	0.2 ppm	0.5 ppm	1 ppm	0.2 ppm	0.5 ppm	1 ppm	0.2 ppm	0.5 ppm	1 ppm
pH 10	0	0	0	0	0	0	0	0	0
pH 9	10	0	0	0	0	0	$8.9 \times 10^3$	0	0
pH 8	$1.1 \times 10^4$	43	1	$9.1 \times 10^3$	2	1	$2.1 \times 10^4$	$10 \times 10^3$	$2.0 \times 10^2$
pH 7	$5.3 \times 10^4$	$3.7 \times 10^3$	$3.6 \times 10^2$	$3.6 \times 10^4$	$9.4 \times 10^3$	$5.6 \times 10^2$	$8.2 \times 10^4$	$6.4 \times 10^3$	$2.5 \times 10^2$
pH 6	$9.8 \times 10^3$	$1.1 \times 10^3$	$2.1 \times 10^2$	$2.1 \times 10^4$	$6.2 \times 10^3$	$3.1 \times 10^2$	$6.1 \times 10^4$	$5.1 \times 10^3$	$1.9 \times 10^2$
pH 5	$5.5 \times 10^3$	$8.2 \times 10^3$	27	$8.7 \times 10^3$	$7.2 \times 10^2$	50	$9.1 \times 10^3$	$2.6 \times 10^3$	$1.1 \times 10^2$
pH 4	$4.2 \times 10^3$	77	3	$3.6 \times 10^3$	12	0	$4.5 \times 10^3$	610	0
pH 3	10	0	0	2	0	0	87	0	0

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Subsequently, using acidic silver-ionic water, tomatoes were cultivated according to hydroponics under the following conditions:

Kind of tomato: Ponderoso

Cultivation room: In a glass room not heated.

Medium: Rock wool; culture solution circulation system.

Culture solution: An aqueous solution obtained by diluting "Bioaqua" (trademark; available from Sanraiku K.K. to 1/3000 with purified water.

Ag<sup>+</sup> ionic water: An aqueous solution of pH 2.4, diluted to 1/2000. Water was fed for 10 minutes every two hours in twenty-four hours.

Those cultivated without using the silver-ionic water were used as a control. Properties of the both culture solutions are shown in Table 8. The number of mature berries, average days for maturation, and longest term for maturation of the tomatoes cultivated by use of both the culture solutions are shown in Table 9.

It is seen from Tables 7 and 8 that the maturation of tomatoes is promoted by the acidic silver-ionic water.

TABLE 8

Properties	Purified water + Bioaqua	Silver-ionic water + Bioaqua
pH	7.19	5.0
EC	0.00	0.64
mu	342	638
DO	9.1	21.0

TABLE 9

Planted (April 5th)	Number of mature berries	Average days for maturation (day)	Longest term for maturation (day)
Purified water + Bioaqua; diluted to 1/1500	44	58.5	73
Silver-ionic water + Bioaqua; diluted to 1/2000	57	50.1	54

Further, using alkaline silver-ionic [Ag(OH)<sub>2</sub><sup>-</sup>, Ag<sub>2</sub>(OH)<sup>+</sup>, etc.] water (pH: 8; silver ion concentration: 50 ppb), radishes *Kaiware* were cultivated under the following conditions:

Kind: Radish *Kaiware*

Cultivation room: wooden dark room of 50 cm wide, 2 m and 50 cm long and 1 m high.

Light source: Fluorescent lamp, 40W×100V (one).

Medium: Urethane cotton.

Water supply: Showered for 30 seconds every 2 hours and 30 minutes according to a ceiling shower system.

Seedling culture box: Made of plastics, provided with drainage holes bored over the whole face of the bottom.

Seedling culture period: 4 days.

For comparison, similar cultivation was carried out with use of general city water (pH 7) and alkaline ionic water (pH 8; containing no silver-ionic water). The germination rate, yield, state of rot of roots were examined for each group. Results obtained are shown in Table 10.

From Table 10, the alkaline silver-ionic water is found to improve the germination rate and yield, and also lessen the rot of roots.

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TABLE 10

	Amount of light (lux)	Germination rate (%)	Yield (g)	Rot of roots
General city water (pH 7)	1100	75	750	Pulpy state
Alkaline ionic water (pH 8)	1100	75	800	Pulpy state
Ag(OH) <sub>2</sub> <sup>-</sup> , Ag <sub>2</sub> (OH) <sup>+</sup> , etc. ionic water (pH 8)	1100	96	1100	None

The silver-ionic water obtained by the apparatus of the present invention can be utilized, for example, in preservation invention can be utilized, for example, in preservation of food, sterilization of tableware or kitchens, sterilization of the inside of refrigerators, etc. There can be also expected bacteriostatic effects on noodles and fish-paste products such as *kamaboko* (boiled fish paste), or effects of preventing spoilage of food by washing food such as meat and vegetables. Use in activation of plants is further expected, suggesting a possibility of wide utilization.

As described above, according to the first embodiment of the present invention, silver ions are dissolved out in the first electrolytic cell chamber, and the water containing silver ions is led into the second electrolytic cell chamber to be formed into acidic silver-ionic water or alkaline silver-ionic water with desired silver ion concentration and pH by controlling valves and by controlling voltage and electric current. Moreover, the acidic or alkaline silver-ionic water can be produced without formation of silver colloids even under relatively higher silver ion concentration. After the silver ions are dissolved out in the first electrolytic cell chamber, the water containing silver ions is led to the anode compartment of the second electrolytic cell chamber, whereby it is possible to increase the electrochemical potential to obtain acidic silver-ionic water having higher electric conductance.

The second preferred embodiment of the present invention will be described below in detail.

In the process for producing silver-ionic water according to the second embodiment of the present invention, the water to be used is first subjected to demineralization treatment in step a). In general, water such as city water and well water contains ionic substances including cations such as Ca<sup>2+</sup> ions, Mg<sup>2+</sup> ions and Na<sup>+</sup> ions, and anions such as HCO<sup>-</sup> ions, SO<sub>4</sub><sup>-</sup> ions, Cl<sup>-</sup> ions and silica-colloidal organic acids. Thus, as will be also seen from its electric conductance which is 80 to 450 μS/cm<sup>3</sup>, components in water can not be uniform and no homogeneous water is obtainable. In order to obtain stable and homogeneous silver-ionic water, it is required to make uniform the components of water. For example, it is necessary to pass water through ion-exchange materials such as ion-exchange resins to decrease cations and anions to a certain level, thereby making uniform the components of water. Here, the water having been subjected to ion-decreasing treatment is desired to have an electric conductance of 30 to 150 μS/cm<sup>3</sup>, more preferably 40 to 80 μS/cm<sup>3</sup>. The water having an electric conductance of less than 30 μS/cm<sup>3</sup> after the ion-decreasing treatment may cause inconveniences such that necessary trace elements can

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not reach the necessary amounts, and that, since an organic carboxylic acid is added to facilitate the electrolysis, sourness may become strong if it is attempted to obtain the desired EC value by increasing the amount of the organic carboxylic acid. The water having an electric conductance of more than  $150 \mu\text{U}/\text{cm}^3$  after the ion-decreasing treatment may cause an inconvenience such that  $\text{Cl}^-$  react with  $\text{Ag}^+$  to make the water cloudy with formation of colloids, causing precipitation.

To the water thus demineralized, an organic carboxylic acid is next added in step (b). As mentioned above, removal of ions in water may cause a lowering of the electric conductance, to make it difficult to smoothly proceed with the electrolysis if no countermeasure is taken. Accordingly, an ionizing compound is required to be added to increase the electric conductance of the water and to facilitate the electrolysis. In the process of the present invention, an organic carboxylic acid, particularly preferably acetic acid, is used as the ionizing compound. The organic carboxylic acid, for example, acetic acid, is ionized in water to give a carboxylic group ion  $\text{CH}_3\text{COO}^-$  and a hydroxyl group ion  $\text{H}^+$ . These ions may not cause precipitation reaction for silver ions produced by electrolysis in the subsequent step (c), and yet can act on the silver ions in the final step (e) to contribute the formation of a complex. Such an organic carboxylic acid may be added to the ion-decreased water preferably in an amount of 0.01 to 0.4% by weight, most preferably about 0.06% by weight, based on the water.

The amount less than 0.01% by weight, of the organic carboxylic acid to be added may cause an inconvenience that the conditions for the electrolysis can be controlled only with difficulty, and the amount more than 0.4% by weight, of the organic carboxylic acid to be added may cause an inconvenience that the silver-ionic water may have an acid taste.

The water to which the organic carboxylic acid has been thus added is then led to step (c) and, under application of a voltage, passed through the first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode, where silver ions are dissolved out in the water. Subsequently, the water containing silver ions is led to the next step (d) and, under the application of a voltage, passed through the second electrolytic cell chamber containing an anode and a cathode and separated into an anode compartment and the cathode compartment with a diaphragm formed between both of said electrodes, and it is important that at least the ionic water passed through the cathode compartment is led to the next step. In the water thus taken out from the cathode compartment,  $\text{Ag}(\text{OH})_2^-$  ions,  $\text{CH}_3\text{COO}^-$  ions, etc. are presumed to have been dissolved. The water obtained from the first electrolytic cell chamber and containing silver ions may otherwise be passed simultaneously through both the anode compartment and cathode compartment of the second electrolytic cell chamber. In this instance, acidic silver-ionic water such as  $\text{Ag}^+$  ionic water can be obtained from the anode compartment. However, when it is unnecessary to produce the water in which acidic ions such as  $\text{Ag}^+$  have been dissolved, ordinary untreated water may be led to the anode compartment and flowed out of the system as it is. In the electrolysis steps as mentioned here, a desired silver ion concentration can be attained by controlling the voltage, electric current and flow rate of water in the first electrolytic cell chamber. There also occurs no formation of colloids of silver

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particles even if the silver ion concentration is increased. This is because the silver ion concentration is controlled in the first electrolytic cell chamber, and the water having thus been made silver-ionic is made more stable in the second electrolytic cell chamber.

The alkaline silver-ionic water thus obtained, in which  $\text{Ag}(\text{OH})_2^-$  ions,  $\text{CH}_3\text{COO}^-$  ions, etc. have been dissolved, is next led into a treatment bath in step (e), where  $\text{NaCl}$  is added in a given amount. The  $\text{NaCl}$  added may react with the alkaline silver ions such as  $\text{Ag}(\text{OH})_2^-$  ions and  $\text{CH}_3\text{COO}^-$  ions, whereby silver is converted into a soluble complex and the pH of the water once falls to pH 3.8, and gradually rises until it reaches about pH 4.2. The mechanism of this reaction has not been made clear in detail, but, it is presumed that complexes such as  $\text{AgCH}_3\text{COO}^-$ ,  $\text{AgCl}_2^-$ ,  $\text{AgCl}_3^{2-}$ , etc. are formed in the water and these are stably dissolved in it. The amount of  $\text{NaCl}$  to be added in step (e) may be preferably 1 to 10% by weight based on the water. The amount less than 1% by weight may cause an inconvenience that clouding and precipitation due to  $\text{AgCl}$  may be caused to form colloid particles, and the amount more than 10% by weight may cause an inconvenience that precipitation due to an excessive chlorine amount may be caused.

The silver-ionic water thus obtained, which contains silver dissolved in the water in the form of complexes such as  $\text{AgCH}_3\text{COO}^-$ ,  $\text{AgCl}_2^-$  and  $\text{AgCl}_3^{2-}$ , etc., may hardly cause the coagulation or precipitation and can be stably preserved for a long period of time. Moreover, it was made clear that, although the monovalent silver ions contained in the conventional silver-ionic water is usually unstable and may react by the action of light to cause halogenation, the silver-ionic water obtained according to the present invention may not cause any reaction at all even by irradiation of light, and can be stably preserved for a long period of time. Also, as compared with the conventional silver-ionic water in which  $\text{Ag}^+$  ions,  $\text{Ag}(\text{OH})_2^-$  ions, etc. are dissolved, the bacteriostatic action has been made stronger. More specifically, when, for example, the silver-ionic water obtained in the present invention is administered in a living body, it can be harmonized with the large quantity of  $\text{Cl}^-$  ions in the extracellular fluid in the body, along which it reaches target cell membranes or bacteria while retaining the state of complex salts, thereby bringing about a higher bacteriostatic action even with lower concentration. Also, the silver-ionic water obtained according to the present invention can have remarkable effects against bacteria present under the water content of about 30% in food, and can achieve an excellent bacteriostatic effect under the concentration quite harmless to human bodies. Still also, the silver-ionic water obtained according to the present invention contains the organic carboxylic acid, and, as the organic carboxylic acid has an action to germinate sporular bacteria, can advantageously have the bacteriostatic action on the sporular bacteria immediately after germination.

In the apparatus for carrying out the process for producing silver-ionic water according to the second embodiment of the present invention, there may be formed at least a flow path in which the water is passed from the ion-exchange chamber through the first mixing bath, the first electrolytic cell chamber, the cathode compartment of the second electrolytic cell chamber, and the second mixing bath, and then flowed out. However, in an apparatus for simultaneously producing the



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acidic silver-ionic water as mentioned above, there may be also provided a flow path in which the water is passed from the ion-exchange chamber through the first mixing bath, the first electrolytic cell chamber, and the anode compartment of the second electrolytic cell chamber, and then flowed out, also, in an apparatus that do not produce such acidic silver-ionic water, there may be additionally provided a flow path in which the water is passed through the anode compartment of the second electrolytic cell chamber and flowed out. As a matter of course, it is also possible to provide both of these flow paths in one apparatus.

FIG. 2 illustrates an example of the apparatus for producing silver-ionic water according to the second embodiment of the present invention.

This apparatus chiefly comprises an ion-exchange chamber 11, a first mixing bath 12, a first electrolytic cell chamber 13, a second electrolytic cell chamber 14, and a second mixing bath 15.

In this apparatus, a first water pipe 16 is connected to the ion-exchange chamber via a flowmeter 17 on the way of the pipe. The ion-exchange chamber 11 comprises an anion-exchange compartment 11a and a cation-exchange compartment 11b, in the insides of which anion-exchange resins and cation-exchange resins are filled, respectively.

This ion-exchange chamber 11 is connected to the first mixing bath 12 by means of the first water pipe 16, on the way of which there is provided an EC (electric conductance) control detector 18. To the first mixing bath 12, an acetic acid-leading pipe 20 is extended from an acetic acid solution tank 19 via a measuring pump 21 on the way of the pipe. The first mixing bath 12 is further connected to the first electrolytic cell chamber 13 through the first water pipe 16, on the way of which there is provided another EC control detector 18.

The first electrolytic cell chamber 13 is enclosed by a bottom plate 22 comprising a non-conductive material, a cathode plate 23 surrounding the outer periphery and made of stainless steel or the like, and a cover plate 24 made of a non-conductive material. Through the cover plate 24, an anode rod 25 is inserted in the manner that the lower end portion thereof may be inward extended. Also, the anode rod 25 is provided with silver or silver alloy 26. To the cover plate 24, connecting pipes 27 and 28 are connected so that the water in the first electrolytic cell chamber 13 can be led out of it. The connecting pipe 27 is provided with a valve 29 and the connecting pipe 28 is provided with a valve 30.

On the other hand, the second electrolytic cell chamber 14 is enclosed by a bottom plate 31 made of a non-conductive material, a cathode plate 32 surrounding the outer periphery and made of stainless steel or the like, and a cover plate 33 made of a non-conductive material. To the cover plate 33, an anode plate 34 is fixed in the manner that it may be inward extended. In the inside of the second electrolytic cell chamber 14, a cylindrical diaphragm 35 is arranged so as to surround the anode plate 34, which diaphragm 35 partitions an anode compartment 36 and a cathode compartment 37. The diaphragm 35 has properties that it allows cations to pass from the anode compartment 36 to the cathode compartment 37 and allows anions to pass from the cathode compartment 37 to the anode compartment 36, to prevent reverse flowing of them.

The aforesaid connecting pipes 27 and 28 are connected to the bottom plate 31, of which the connecting pipe 28 is led to the above cathode compartment 37 and

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the connecting pipe 28 is led to the above anode compartment 36. Lead-out pipes 38 and 39 are connected to the cover plate 33, of which the lead-out pipe 38 is led to the cathode compartment 37 and the lead-out pipe 39 is led to the anode compartment 36. The lead-out pipes 38 and 39 are provided with valves 40 and 41, respectively.

A second water pipe 42 is connected between the valve 29 of the connecting pipe 27 and the bottom plate 31 via a flowmeter 43 and a valve 44. A third water pipe 45 is connected between the valve 30 of the connecting pipe 28 and the bottom plate 31 via a flowmeter 46 and a valve 47.

On the other hand, a second mixing bath 15 used for adding NaCl is provided at a lower portion of the outlet 48 of the lead-out pipe 38, and another lead-out pipe 49 is connected to a lower part of the second mixing bath, on the way of which is provided a valve 50. To the second mixing bath 15, an NaCl-feeding pipe 52 extends from an NaCl solution tank 51 via a measuring pump 53 provided on the way of the pipe.

To obtain silver-ionic water by use of this apparatus, water is led to the ion-exchange chamber 11 through the first water pipe 16, where anions and cations present in the water is removed to a give value and the electric conductance in the water is made to be 30 to 150  $\mu\text{S}/\text{cm}^3$ . The water demineralized in this manner is flowed out from the ion-exchange chamber to the first water pipe 16 and flowed into the first mixing bath 12 while being midway controlled by the EC control detector 18 to see whether or not the electric conductance is in a given value. To the first mixing bath 12, a predetermined amount of acetic acid is added through the acetic acid-feeding pipe from the acetic acid solution tank 19, and mixed into the water in the concentration of 0.01 to 0.4% by weight. The water to which the acetic acid has been added in this manner is led to the first electrolytic cell chamber 13 through the first water pipe 16, while being midway controlled by the EC control detector 18 to see whether or not the electric conductance is in a given value.

Here, to also simultaneously obtain acidic silver-ionic water by use of this apparatus, valves 29, 30, 40 and 41 may be opened and valves 44 and 47 may be closed, so that water is fed from the first water-feeding pipe 16. The water thus fed from the first water-feeding pipe 16 is led into the first electrolytic cell chamber 13, where silver ions are formed. Then, part of the water containing silver ions is led into the anode compartment 36 of the second electrolytic cell chamber 14 through the connecting pipe 28, and taken out from the lead-out pipe 39 in the form of acidic silver-ionic (e.g.  $\text{Ag}^+$ ) water formed in the anode compartment 36 and having an electric conductance of 100 to 2000  $\mu\text{S}/\text{cm}^3$ . At the same time therewith, remaining part of the water containing silver ions is led into the cathode compartment 37 of the second electrolytic cell chamber 14 through the connecting pipe 27, and taken out from the lead-out pipe 38 in the form of alkaline silver-ionic water in which  $\text{Ag}(\text{OH})_2^-$  ions,  $\text{CH}_3\text{COO}^-$  ions, etc. are dissolved in the cathode compartment 37.

In connection with this, in the case the flow rate of water flowing through the first electrolytic cell chamber 13 was made to be 4.5 lit/min, the relationship between the electric power consumption and the silver ion concentration in the first electrolytic cell chamber 13 is shown in Table 11. Also, the relationship between the voltage and electric current in the second electro-



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lytic cell chamber, the flow rate of the water flowing in the cathode compartment 37 of the second electrolytic cell chamber 14, and the pH of the water taken out from the cathode compartment 37 of the second electrolytic cell chamber 14 is shown in Table 12.

TABLE 11

Electric power consumption	Silver ion concentration
9.4 W	1,000 ppb
36.0 W	1,940 ppb

TABLE 12

pH value	Flow rate (lit/min)	Voltage	Electric current
3.8	4.6	100 V	10 A

The water in which  $\text{Ag}(\text{OH})_2^-$  ions,  $\text{CH}_3\text{COO}^-$  ions, etc. are dissolved in the cathode compartment 38 is flowed into the second mixing bath 15. On the other hand, a predetermined amount of NaCl is flowed thereinto from the NaCl solution tank 51 through the NaCl-feeding pipe 52. Accordingly, the above water is mixed with 0.01 to 0.4% by weight of NaCl in the second mixing bath 51, where  $\text{Na}^+$  ions,  $\text{Cl}^-$  ions and so forth react with  $\text{Ag}(\text{OH})_2^-$  ions,  $\text{CH}_3\text{COO}^-$  ions and so forth in the water to form complexes such as  $\text{AgCH}_3\text{COO}^-$ ,  $\text{AgCl}_2^-$ ,  $\text{AgCl}_3^{2-}$  and so forth. Along with this course, the pH of the water once falls to pH 3.8, and gradually rises until it reaches about pH 4.2. After silver complexes was formed in this manner, the valve 50 may be opened to take out desired silver-ionic water from the lead-out pipe 49.

## TEST EXAMPLE 2

Using the above apparatus according to the second embodiment of the present invention, acidic silver-ionic water each having the pH of 4.2 and a silver ion concentration of 30 ppb, 100 ppb, 500 ppb, 2,000 ppb and 1 ppm was prepared. Thereafter, a platinum loop each of *Escherichia coli* and *Staphylococcus* (concentration:  $10^5/\text{cm}^3$ ) was inoculated into each solution, followed by addition of a 0.1% broth, and the movements (viable cell counts) of the bacteria were observed with time lapse while culturing them at 35° C. As a result, the viable cell counts on *Escherichia coli* were as shown in Table 13.

As to *Staphylococcus*, the state of growth thereof was observed based on the turbidity of the solutions to obtain the results as shown in Table 14. Thus, the acidic silver-ionic water was found to have a remarkable bacteriostatic action.

The *Escherichia coli* and *Staphylococcus* used in the above were further cultured to repeat experiments similar to the above several times, but there appeared no bacteria resistant to silver ions, showing the same bacteriostatic action as in the first instance. Thus, there is a feature that the appearance of resistant bacteria, which has been very usual in using conventional medicaments, is not seen at all in using this silver ions.

TABLE 13

Contact time	Silver ion concentration					
	Control 0	30 ppb	100 ppb	500 ppb	2000 ppb	1 ppm
10 min	2000	160	100	0	0	0
2 hrs	3500	40	20	0	0	0
4 hrs	20000	10	0	0	0	0

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TABLE 13-continued

Contact time	Silver ion concentration					
	Control 0	30 ppb	100 ppb	500 ppb	2000 ppb	1 ppm
6 hrs	55000	0	0	0	0	0
24 hrs	19000000	0	0	0	0	0
48 hrs	80000000	0	0	0	0	0

TABLE 14

Contact time	Control	30 ppb	100 ppb	500 ppb	2000 ppb	1 ppm
24 hrs	+	+	-	-	-	-
48 hrs	+	+	-	-	-	-
72 hrs	+	-	-	-	-	-

Subsequently, using the silver-ionic water, tomatos were cultivated according to hydroponics under the following conditions:

Kind of tomato: Ponderozer

Cultivation room: In a glass room not heated.

Medium: Rock wool; culture solution circulation system.

Culture solution: An aqueous solution obtained by diluting "Bioaqua" (trademark; available from Sanraiku K.K. to 1/3000 with purified water.

Silver-ionic water: An aqueous solution of pH 3 diluted to 1/2000. Water was fed for 10 minutes every two hours in twenty-four hours.

Those cultivated without using the silver-ionic water were used as a control. Properties of the both culture solutions are shown in Table 15. The number of mature berries, average days for maturation, and longest term for maturation of the tomatos cultivated by use of both the culture solutions are shown in Table 16.

Thus, it is seen that the maturation of tomatos is promoted by the acidic silver-ionic water obtained by the process according to the present invention.

TABLE 15

Properties	Purified water + Bioaqua	Silver-ionic water + Bioaqua
pH	7.19	6.5
EC	0.46	0.64
mg/l	342	638
DO	9.1	21.0

TABLE 16

Planted (April 5th)	Number of mature berries	Average days for maturation (day)	Longest term for maturation (day)
Purified water + Bioaqua; diluted to 1/1500	44	58.5	73
Silver-ionic water + Bioaqua; diluted to 1/2000	57	50.1	54

Further, to know the bactericidal effect of the silver-ionic water obtained according to the production process of the present invention, a spore test was carried out in the following manner: after heated for 10 minutes using hot water of 90° C., 0.06% by weight of acetic acid was added to silver-ionic water on the anode side of an electrolyte, and the mixture was added to *B. Cereus* detected from buckwheat flour, which was then

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cultured in a YCC broth at 37° C. for 24 hours before the germination was recognized. Similar cultivation was also carried out in respect of *B. subtilis* (a spore suspension available from Daiichi Kagaku Yakuhin K.K.). To know bactericidal effect without heating after the above germination was recognized, silver-ionic water having a silver ion concentration of 100 ppb and 1 ppm each, obtained by the production process according to the second embodiment of the present invention, was added to, and brought into contact with, the broth having bacterial concentration of  $10^5$  to  $10^6$  bacteria/ml, and thereafter the viable cell counts were examined. Results obtained are shown in Table 17.

Thus, the silver-ionic water obtained by the production process according to the present invention was found to have a remarkable bactericidal action against *B. cereus* and *B. subtilis*.

TABLE 17

Tested bacteria	Silver ion concentration		
	100 ppb	500 ppb	1 ppm
<i>B. subtilis</i>	$10^3$	15	0
<i>B. cereus</i>	$10^3$	0	0

Still further, to know the bactericidal effect against thermal resistant bacteria, of the silver-ionic water obtained by the production process of the present invention, tests were carried out in the following manner: First, into a powdery material comprising 30% of buckwheat flour and 70% of wheat flour, 30% of additive water (well water) was poured to prepare soba (buckwheat). Using various kinds of silver-ionic water as water for boiling, the buckwheat was boiled at 100° C. for 3 minutes, and the bactericidal effect against *B. cereus* cultivated at 37° C. for 24 hours was examined. As a result, as shown in Table 18, the silver-ionic water according to the present invention showed most remarkable effects.

As described in the foregoing, according to the second embodiment of the present invention, water is beforehand subjected to a demineralization treatment; and organic carboxylic acid is added thereto; thereafter silver ions are dissolved out in the first electrolytic cell chamber, which is led to the cathode compartment of the second electrolytic cell chamber; NaCl is further added thereto; whereby water soluble silver ion complexes can be formed. Accordingly, it is possible to readily produce silver-ionic water that can stably retain the solubilized state for a long period of time and can show strong effect on the intended action with lower concentration.

TABLE 18

Water for boiling	Silver ion concentration		
	200 ppb	500 ppb	1 ppm
Alkaline silver-ionic water + COOH	$8.2 \times 10^5$	$2.1 \times 10^4$	$1.8 \times 10^3$
Acidic silver-ionic water + COOH	$5.7 \times 10^6$	$4.1 \times 10^5$	$1.1 \times 10^4$
Alkaline silver-ionic water + COOH + NaCl	$3.1 \times 10^4$	$2.1 \times 10^3$	60
Acidic silver-ionic water + COOH + NaCl	$6.2 \times 10^5$	$3.2 \times 10^4$	$4.5 \times 10^3$
Control (well water)	$5.6 \times 10^7$		

What is claimed is:

1. A process for producing silver-ionic water, comprising;
  - a step of passing water through a first electrolytic cell chamber containing an anode and a cathode and

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provided with silver on the anode, under the application of a voltage, to dissolve out silver ions; and a step of passing the water in which said silver ions have been dissolved out, through either one or both of an anode compartment and a cathode compartment of a second electrolytic cell chamber containing an anode and a cathode and separated into said anode compartment and said cathode compartment with a diaphragm formed between both of said electrodes;

said diaphragm allowing cations to pass from the anode compartment to the cathode compartment and allowing anionic ions to pass from the cathode compartment to the anode compartment, whereby the water passed from the first electrolytic cell chamber through the anode compartment of the second electrolytic cell chamber turns to acidic silver-ionic water and the water passed from the first electrolytic cell chamber through the cathode compartment of the second electrolytic cell chamber turns to alkaline silver-ionic water.

2. The process according to claim 1, wherein said acidic silver-ionic water has the pH of 5 or less and an electric conductance of 350 to 2000  $\mu$ /cm<sup>3</sup> and said alkaline silver-ionic water has the pH of 8 or more.

3. An apparatus for producing silver-ionic water, comprising;

- a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode,
  - a second electrolytic cell chamber containing an anode and a cathode and separated into an anode compartment and a cathode compartment with a diaphragm formed between both of said electrodes;
  - a least one of a flow path in which water is passed from said first electrolytic cell chamber through anode compartment of said second electrolytic cell chamber and flowed out, and a flow path in which water is passed from said first electrolytic cell chamber through the cathode compartment of said second electrolytic cell chamber and flowed out;
- said diaphragm allowing cations to pass from the anode compartment to the cathode compartment and allowing anionic ions to pass from the cathode compartment to the anode compartment, whereby the water passed from the first electrolytic cell chamber through the anode compartment of the second electrolytic cell chamber turns to acidic silver-ionic water and the water passed from the first electrolytic cell chamber through the cathode compartment of the second electrolytic cell chamber turns to alkaline silver-ionic water.

4. The apparatus according to claim 3, wherein said apparatus further comprises a flow path in which the water is directly passed through said anode compartment of the second electrolytic cell chamber and flowed out, and another flow path in which the water is directly passed through said cathode compartment of the second electrolytic cell chamber and flowed out.

5. A process for producing silver-ionic water, comprising;

- (a) subjecting water to a demineralizing treatment to decrease ionic substances in the water;
- (b) adding an organic carboxylic acid to the ion-decreased water;
- (c) passing the water to which the organic carboxylic acid has been added, through a first electrolytic

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- cell chamber containing an anode and a cathode and provided with silver on the anode, under the application of a voltage, to dissolve, out silver ions;
- (d) passing under the application of a directcurrent voltage the water in which said silver ions have been dissolved out, through at least a cathode compartment side of a second electrolytic cell chamber containing an anode and a cathode and separated into an anode compartment and the cathode compartment with a diaphragm formed between both of said electrodes; and
- (e) adding sodium chloride to the water having been passed through said cathode compartment so that silver may be present in the water as a soluble complex;
- said diaphragm allowing cations to pass from the anode compartment to the cathode compartment and allowing anionic ions to pass from the cathode compartment to the anode compartment, whereby the water passed from the first electrolytic cell chamber through the anode compartment of the second electrolytic cell chamber turns to acidic silver-ionic water and the water passed from the first electrolytic cell chamber through the cathode compartment of the second electrolytic cell chamber turns to alkaline silver-ionic water.
6. The process according to claim 5, wherein said water in step (a) is demineralized to have an electric conductance of 30 to 150  $\mu\text{S}/\text{cm}^3$ .
7. The process according to claim 5, wherein said organic carboxylic acid is acetic acid.
8. The process according to claim 5, wherein said carboxylic acid is added in an amount of 0.01 to 0.4% by weight based on said water in step (b).
9. The process according to claim 5, wherein said sodium chloride is added in an amount of 1 to 10% by weight based on said water in step (e).
10. An apparatus for producing silver-ionic water, comprising;
- an ion exchange chamber filled with an ion exchange material;
- a first mixing bath having an organic carboxylic acid feeding means;

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- a first electrolytic cell chamber containing an anode and a cathode and provided with silver on the anode;
- a second electrolytic cell chamber having an anode and a cathode and separated into an anode compartment and a cathode compartment with a diaphragm formed between both of said electrodes; and
- a second mixing bath having sodium chloride feeding means;
- in such a juxtaposition that a water-feeding pipe is connected to an inlet of the ion exchange chamber, an outlet of the ion exchange chamber is connected to an inlet of the first mixing bath, an outlet of the first mixing bath is connected to an inlet of the first electrolytic cell chamber, an outlet of the first electrolytic cell chamber is separately connected to the anode compartment and cathode compartment of the second electrolytic cell chamber, the cathode compartment of the second electrolytic cell chamber is connected to the second mixing bath, a pipe for feeding additional water is connected to the flow path connecting the first electrolytic cell chamber and the anode compartment in the second electrolytic cell chamber, and a valve is provided for selectively flowing the water passed through the first electrolytic cell chamber or the additional water, into the anode compartment of the second electrolytic cell chamber, to form a flow path in which water is flowed out after being passed from said ion exchange chamber through said first mixing bath, said first electrolytic cell chamber, the cathode compartment of said second electrolytic cell chamber and said second mixing bath;
- said diaphragm allowing cations to pass from the anode compartment to the cathode compartment and allowing anionic ions to pass from the cathode compartment to the anode compartment, whereby the water passed from the first electrolytic cell chamber through the anode compartment of the second electrolytic cell chamber turns to acidic silver-ionic water and the water passed from the first electrolytic cell chamber through the cathode compartment of the second electrolytic cell chamber turns to alkaline silver-ionic water.
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[Labdev J. Sci. Tech., Vol. 8-B No. 4, Oct. 1970]

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## Development of Ready to Use Antiseptic Dressings—Part I : Development of Surgical Gauze and Absorbent Cotton by Using In Vitro Laboratory Evaluation Methods.

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### ABSTRACT

Surgical gauze and absorbent cotton treated with a combination of cetyl dimethyl benzyl ammonium chloride (Acinol CDMB) and ammoniacal silver chloride have been found to possess good antibacterial activity against *S. aureus*, *P. pyocyanea*, *Strept. pyogenes*, *E. coli*, *S. albus*, *B. subtilis*, *Salm. typhi*, and *Strept. faecalis*. It retains its antibacterial activity for atleast one year. The colour of the gauze is not affected by treatment. It does not irritate skin or produce untoward effects on artificially produced wounds in animals.

Surgical gauze treated with silver citrate showed good bacteriostatic activity but had no bactericidal activity.

### Introduction

The work was undertaken to produce sterile, antiseptic surgical dressings suitable for medical use. Such dressings could offer ready protection to human wounds in field areas where proper facilities for treatment are usually lacking and in hospital outdoors.

Commercially available Field and Shell dressings, at present used by services are treated with low concentration of acriflavine. These dressings and surgical gauze given a treatment with proflavine have been tested for antibacterial property in the present study. Based on the reported literature that ammoniacal silver nitrate, silver citrate (Sykes 1965 a), cetyl dimethyl benzyl ammonium chloride (Acinol CDMB) (Rao *et al.* 1967), a cheap quaternary ammonium compound manufactured by M/s Ahura Chemical Ltd., Bombay, possess good antibacterial activity, attempts have been made in the present investigation to prepare

sterile, antiseptic surgical dressings by impregnation of surgical gauze and absorbent cotton with such antibacterial compounds. Use of antibiotics has purposely been avoided for their skin sensitization possibility and their poor storage qualities.

### Material and methods

Treatments that were given to surgical gauze for imparting the desired property are mentioned below. In each case 10 gm of gauze was thoroughly dipped in 100 ml of aqueous solution, squeezed with hand and air dried.

1. 1% Acinol CDMB 100 in water
2. 0.4% Ammoniacal silver chloride
3. 0.4% Ammoniacal silver chloride alongwith 1% Acinol CDMB 100
4. 0.8% Ammoniacal silver chloride alongwith 1% Acinol CDMB 100
5. 1.6% Ammoniacal silver chloride alongwith 1% Acinol CDMB 100
6. 2.4% Ammoniacal silver chloride alongwith 1% Acinol CDMB 100
7. 0.5% Silver citrate
8. 0.5% proflavine

Following treatment was given to cotton dressing:

- (A) A solution of 0.4% ammoniacal silver chloride and 1% Acinol CDMB 100 in water was applied by a fines pray on the surface of sterilized cotton pads and allowed to dry in air. Cotton pads thus obtained were wrapped in treated gauze (treatment 3) and packed in brown paper bags, sealed and heated in a drying oven at 160°C for 1 hour. These brown paper bags, containing the cotton dressings, were put in polythene bags and sealed.

Surgical gauze given treatment 3 was also kept in artificial perspiration solution for 72 hours, air dried and its antibacterial activity studied on agar plate. The composition of artificial perspiration solution used was as follows:

Urea — 0.071% }  
NaCl — 0.50% } Adjusted to pH 3.8

To find out the resistance of the antibacterial property to washing gauze given treatment 3 was scrubbed well in soap solution, washed under tap water and air dried.

Details of bacteriological studies carried out with the test samples are described below:-

**Organisms used:** in the study are (1) *Staphylococcus aureus*, (2) *Staphylococcus albus*, (3) *Streptococcus faecalis*, (4) *Streptococcus pyogenes* (5) *Bacillus subtilis* (6) *Escherichia coli*, (7) *Pseudomonas pyocyanea*, (8) *Salmonella typhi*;

**Nutrient medium and nutrient agar:** were prepared by the method specified in B. P. 1963.

#### Preparation of culture suspension/plate

Culture suspension of the test organism was prepared by inoculating a loopful of the growth from a nutrient agar slant into 10 ml of sterile nutrient medium in a test tube. This was incubated at 37°C for 24 hours (IC). 0.2 ml of this culture suspension was inoculated into 25 ml of molten nutrient agar in petri dishes and allowed to cool and solidify (SC).

#### Antibacterial activity

Four small pieces of the treated gauze sample, to be tested, were moistened with sterile water and placed equidistantly in each petri dish (SC). These plates were then incubated at 37°C for 24 hours and zones of inhibition examined visually at the end of that period and their antibacterial activity assessed.

#### Counting of organisms in a culture suspension

An unknown bacterial concentration contained in 1 ml of culture suspension (IC) was transferred to a conical flask containing 40 ml sterile nutrient medium. 1 ml of this diluted culture suspension was withdrawn and serially diluted to 10 ml in 9 ml of water contained in each one of a series of ten test tubes. One ml of suspension from each of these serial dilutions were transferred respectively to test tubes each containing 20 ml of nutrient agar maintained at 45-50°C. Suspension was thoroughly mixed with nutrient agar and poured serially into petri dishes and allowed to set. After incubation of these petri dishes at 37°C for 24 hours a suitable dish was picked up and its number of separate colonies

counted. Assuming that a single bacterium gave rise to one colony, the number of organisms in the original bacterial suspension was calculated.

#### Bactericidal activity

One g of the treated gauze, to be tested, was dipped in culture suspension of known bacterial count in conical flask, the contents stirred thoroughly and allowed to stand. At intervals of 30 minutes, 1, 2, 4 and 24 hours, 0.2 ml of the solution was withdrawn and added to 10 ml of nutrient medium contained in a test tube. In each case, three replicates were run. Controls were run simultaneously with untreated sterile gauze. The tubes were incubated at 37°C for eight days and bacterial growth was observed visually. Bactericidal activity has been claimed only when all the control tubes showed heavy bacterial growth while the experimental tubes showing none at the end of eight days.

**Sterility test:** The test of sterility of the gauze and cotton pads was done according to B. P. C. 1965 and B. P. 1963.

**Skin irritation test:** was carried out according to the method of Rao *et al.*, 1967.

#### Results and discussion

Table 1 gives the results of the antibacterial activity of different materials tested. It shows that acriflavine in Field and Shell dressings had no antibacterial action against the micro-organisms tried. Proflavine treated surgical gauze (treatment 8) showed good antibacterial action against *Strept. pyogenes*, *S. albus* and *B. subtilis*, but it had no activity against *S. aureus*, *P. pyocyanea*, *E. coli* and *Salm. typhi*. The ineffectiveness of proflavine, an imported compound, against four out of seven test organisms, in addition to the fact that *S. aureus* and *P. pyocyanea* are important in wound infection (Sykes 1965 b) greatly limits the use of proflavine in the field of antiseptic surgical dressings.

Surgical gauze treated with one percent Acinol showed very good antibacterial action against *Strept. pyogenes*, *S. albus*, *B. subtilis*, *S. aureus*. Its antibacterial activity, however, was poor against *E. coli* and *Salm. typhi* and it was inactive against *P. pyocyanea*.

In our search for antiseptic activity against *P. pyocyanea*, treatment of surgical gauze with 0.4% ammoniacal solution of silver chloride in water (treatment 2) has been found effective in imparting good antibacterial activity against *P. pyocyanea*.

Surgical gauze treated with a mixture of 0.4% ammoniacal silver chloride solution and 1% Acinol CDMB 100 in water (treatment 3) had good anti-

TABLE I

Antibacterial activity of treated gauze, cotton and field and shell dressing

Treatment No.	A*	B*	C*	D*	E*	F*	G*	H*
1	+++	Nil to ±	++++	+	+	++++	+++	Not done
2	+	+++	±	+	+	+	+	—do—
3	+++	++	++++	++	++	++++	+++	+
4	+++	++	++++	++	++	++++	+++	+
5	+++	++	++++	++	++	++++	+++	+
6	+++	+++	++++	++	+++	++++	+++	++
7	+++	++++	+++	+++	+++	++++	++++	++
8	Nil	Nil	++	Nil	Nil	++++	+++	Not done
Cotton pads	+++	+	++	++	++	+++	++	+
Field & shell dressings	Nil	Nil	Nil	Nil	Nil	Nil	±	Not done

A\* = *Staph. aureus*; B\* = *P. pyocyanea*; C\* = *Strept. pyogenes*; D\* = *E. coli*; E = *Salm. typhi*; F\* = *S. albus*; G\* = *B. subtilis*; H\* = *Strept. faecalis*.

Antibacterial activity : ++++ = very good; +++ = good; ++ = fair; + = poor; ± = very poor.

TABLE 2

Bactericidal activity of two of the treated samples of surgical gauze

Microorganisms studied	App : bacterial count/ml of medium (out of a total Vol. of 40 ml)	Bacterial growth after incubation for 8 days at 37°C							
		Sample after treatment 3			Sample after treatment 7		Untreated control		
		30 min. contact with plain media	30 min. contact with serum media	60 min. contact with serum media	24 hrs. contact with plain media	24 hrs. contact with serum media	24 hrs. contact with plain media	24 hrs. contact with serum media	
<i>Staph. aureus</i>	8 × 10 <sup>8</sup>	—	—	—	+	+	+	+	
<i>P. pyocyanea</i>	5 × 10 <sup>6</sup>	—	—	—	+	+	+	+	
<i>Strept. pyogenes</i>	5 × 10 <sup>7</sup>	—	—	—	+	+	+	+	
<i>E. coli</i>	13 × 10 <sup>7</sup>	—	+	—	+	+	+	+	
<i>Salm. typhi</i>	5 × 10 <sup>8</sup>	—	—	—	+	+	+	+	
<i>Staph. albus</i>	2 × 10 <sup>7</sup>	—	—	—	+	+	+	+	
<i>B. subtilis</i>	6 × 10 <sup>7</sup>	—	—	—	+	+	+	+	
<i>Strept. faecalis</i>	4 × 10 <sup>6</sup>	—	—	—	+	+	+	+	

In all cases, the sample and nutrient medium containing bacterial cultures were kept in contact for 30 min., 1 hour, 2 hours, 4 hours and 24 hours.

In sample 3, reading for 2, 4 and 24 hours have not been recorded, because there was no growth in these periods. On the other hand observations for 30 min., 1, 2 and 4 hours in sample 7 and control sample have been omitted because even after 24 hours of contact with treatment 7 the organisms multiplied.

(+) indicates growth of organisms; (—) shows no growth.

TABLE 3

## Sterility tests on treated gauze sample

Days of incubation	Sample 3 (tested within 7 days of preparation)		Sample 3 (tested after 1 year of preparation)		Sample 7 (tested within 7 days of preparation)		Control	
	No. of contaminated aerobic tubes	No. of contaminated anaerobic tubes	No. of contaminated aerobic tubes	No. of contaminated anaerobic tubes	No. of contaminated aerobic tubes	No. of contaminated anaerobic tubes	No. of contaminated aerobic tubes	No. of contaminated anaerobic tubes
1	None	None	None	None	5	10	None	None
2	"	"	"	"	6	10	"	"
3	"	"	"	"	7	10	"	"
4	"	"	"	2	7	10	"	"
5	"	"	"	2	7	10	"	"
6	"	"	"	2	7	10	"	"
7	"	"	"	2	7	10	"	"
8	"	"	"	2	7	10	"	"

In each case there were ten aerobic and ten anaerobic tubes.

The test samples were wrapped in brown paper and stored in a dark place. They were not sterilized by heat treatment or by autoclaving.

TABLE 4

## Stability of bacteriostatic property of gauze having treatment 3

Sample treated with	S. aur.	B. subt.	S. albus.	S. pyogenes	E. coli	P. pyo.	S. typhi.	S. facc.
No washing	++	+++	+++	+++	++	++	++	++
One washing	+	+++	++	++	++	+	+	±
Three washing	+	+++	++	++	++	+	—	—
Five washing	—	++	++	++	++	—	—	—
Ten washing	—	++	+	+	+	—	—	—
Heated at 150°C	++	+++	+++	+++	++	++	++	++
Autoclave	++	+++	+++	+++	++	++	++	++
Tested after 5 months	+++	++	+++	+++	++	+	+	+
Tested after 7 months	++	+++	+++	+++	++	+	+	+
Tested after 1 year	++	+++	+++	+++	++	+	+	+
Dipped in artificial perspiration solution for 78 hours	—	++	++	++	++	—	—	—

Antibacterial activity : +++ = good; ++ = fair; + = poor; ± = very poor; — = nil.

For details see text.



bacterial property against *S. pyogenes*, *S. albus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. pyocyanea*, *Salm. typhi* and *Strept. faecalis*.

Increase in the concentration of ammoniacal silver chloride solution in a combination with 1% Acinol (treatment 4 and 5) did not show an appreciable improvement in antibacterial activity. The antibacterial property however was definitely improved when 2.4% ammoniacal silver chloride was used along with 1% Acinol (treatment 6). From the point of view of lower cost and probable toxicity sample 3 was considered to be the best. Cotton pads treated by method (A) showed satisfactory antibacterial action needed for a wound dressing (Table 1).

Surgical gauze treated with 0.5% silver citrate (treatment 7) showed very good antibacterial activity against the organisms studied (Table 1). The antibacterial activity as shown on agar plate was better than that of sample 3.

Ammoniacal silver chloride or silver citrate treated gauze (or cotton) retained their original colour when packed in brown or black paper bags.

Surgical gauze samples with treatments 3 and 7 were subjected to study of bactericidal activity. The results presented in Table 2 show that treatment 3 was strongly bactericidal in plain media or in a media mixed with horse serum while treatment 7 had completely failed to show any bactericidal activity in both the media.

Sterility test during storage was performed with samples 3 and 7 stored in unsealed brown paper wrappings. It will be observed in Table 3 that sample 3 was sterile under ordinary packing condition even after one year. Sample 7 did not maintain sterility even for 7 days.

Thus from the point of view of bactericidal property and sterility to cotton material and its ultimate use in ready-to-use antiseptic dressings, treatment 3 is considered superior to treatment 7.

Stability of the antibacterial property in samples given treatment 3 was studied in greater detail (Table 4). It can be seen from the table that heating at 160°C for 1 hour or autoclaving at 15 lb pressure for 20 minutes did not reduce the antibacterial action. Autoclaving however, brought a deterioration in colour (the colour became bluish), whereas dry heating at 160°C in brown paper packets did not change the colour. Storing upto one year at room temperature in loose unsealed paper packing did not change the antibacterial action except for minor reduction in activity against *P. pyocyanea* and *S. typhi*. When the sample was

dipped in artificial sweat for 72 hours antibacterial property was reduced but it still retained fairly good activity against *B. subtilis*, *S. albus*, *S. pyogenes* and *E. coli*. One washing with thorough soap scrubbing and washing under running tap water, reduces the antibacterial activity to a slight extent and is still active against all the organisms studied. After 3 such rigorous soap washing antibacterial action against *S. typhi* and *S. faecalis* and after five such washings activity against *S. aureus* and *P. pyocyanea* disappeared. After ten such washings activity still persisted against *B. subtilis*, *S. pyogenes*, *S. albus* and *E. coli*. Thus it is noted that antibacterial activity in gauze having treatment 3 is quite stable and is suitable for use after long storage.

When gauze having treatment 3 was kept in contact with artificial wounds for a few days on albino rats no untoward effect was noted.

Thus laboratory evaluation shows that cotton material treated with 0.4% ammoniacal silver chloride and 1% Acinol is suitable for a clinical trial for using as ready-to-use antiseptic dressings on human wounds. It may be pointed out that Ghose and Sengupta 1969 had prepared nontoxic, stable ready-to-use antiseptic surgical gauze suitable for using on wound. It suffered from drawbacks of being black in colour and of low bursting strength. The treatment recommended in this report for preparing nontoxic sterile antiseptic surgical dressings is free from these drawbacks.

#### Acknowledgment

Authors express their sincere thanks to Dr. J. N. Nanda, Director DRL(M), Dr. G. N. Haksar, Officer-In-Charge and Dr. K. B. Sehra, A. D. Drugs & Antibiotics Division for taking interest in the work and to Mr. G. Pandya for rendering technical assistance.

Received : 14 July, 1970

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A01N 55/02, 59/20</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/39837</b> <b>(43) International Publication Date:</b> 19 December 1996 (19.12.96)
<b>(21) International Application Number:</b> PCT/US96/07872 <b>(22) International Filing Date:</b> 30 May 1996 (30.05.96)  <b>(30) Priority Data:</b> 08/486,832 7 June 1995 (07.06.95) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 08/486,832 (CIP) Filed on 7 June 1995 (07.06.95)  <b>(71) Applicant (for all designated States except US):</b> BIO-LAB, INC. [US/US]; 627 East College Avenue, P.O. Box 1489, Decatur, GA 30031 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> GARRIS, John, P. [US/US]; 3690 Alcot Way, Cumming, GA 30131 (US).  <b>(74) Agents:</b> THOMAS, Timothy, N. et al.; Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).		<b>(81) Designated States:</b> AU, CA, GB, NZ, US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SEQUESTERED METAL BIOCIDES USING IONIC POLYMERIC STABILIZING AGENTS  <b>(57) Abstract</b>  Metal-containing biocides for treating swimming pool water, spas, cooling towers, or other industrial applications without staining are disclosed. The biocidal compositions include a source of metal and a polymeric sequestering agent. The polymeric sequestering agent may be a water-soluble anionic or cationic polymer. Conventional sequestering agents may also be included in the composition.		

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**SEQUESTERED METAL BIOCIDES  
USING IONIC POLYMERIC STABILIZING AGENTS**

**FIELD OF THE INVENTION**

5       The present invention relates generally to biocides for water systems such as swimming pools, spas, cooling towers, or other industrial applications, and more particularly to biocides using copper, silver or zinc as their active agent.

**BACKGROUND OF THE INVENTION**

10       Certain metals, such as copper, silver, zinc, etc., are known to possess biocidal qualities when used in circulating water systems. Unfortunately, many metal ions easily precipitate in alkaline or near-alkaline water conditions as insoluble salts of oxides, hydroxides, and/or carbonates, removing the metals from the system and thus removing the  
15       ability of the metal ion to act as a biocide.

      In order to improve the efficacy of metals to act as biocides in water, sequestering agents, such as alkanolamines, aminocarboxylic acids or citric acid have been used to improve the stability of the metal in aqueous  
20       solutions conditions. These simple, organic compounds contain amine, hydroxyl, and carboxyl functionalities that exhibit sequestration capacity for polyvalent metal cations. Problems remain however, because alkanolamines, aminocarboxylic acids, and citric acid deteriorate quickly in  
25       the presence of halogens and other oxidizers, bacteria, sunlight or heat, thus allowing the metal to become unsequestered and subject to loss due to precipitation. The

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net result in pools, spas, cooling towers or other water systems is a loss of activity of the metal ions.

Another problem encountered in water treatment applications, such as swimming pools or spas, is that metals precipitate to form unsightly stains on the pool's surface. In particular, metal hydroxides, metal oxides and metal carbonates are known to cause unsightly stains that are difficult to remove.

A need therefore exists for a method of stabilizing soluble metals for longer periods of time during treatment and application, thereby increasing their effective life and preventing stains from occurring on swimming pool surfaces. The present invention addresses that need.

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## SUMMARY OF THE INVENTION

Briefly describing one aspect of the present invention there are provided metal-containing biocides for treating swimming pool water without staining the sides of the pool.

- 5 The inventive biocidal compositions include a source of a metal such as copper, silver, zinc, etc., and a polymeric sequestering agent. The polymeric sequestering agent may be a water-soluble anionic polymer or a water-soluble cationic polymer. Conventional sequestering agents may also be  
10 included in the composition.

One object of the present invention is to provide metal-containing biocides that do not stain the solid surfaces of a swimming pool.

- 15 A second object of the present invention is to provide a metal-containing biocidal composition that maintains activity longer due to stabilization of metal ions in solution by polymeric sequesterants.

Further objects and advantages of the present invention will be apparent from the following description.

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## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the residence time of soluble copper, copper/polymer and copper triethanolamine using 28 liter aquaria (error bars equal to  $\pm$  one std. deviation).

5        FIGS. 2 and 3 show equivalent residence times for those samples containing copper/TEA and copper/polymer formulations.

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## DESCRIPTION OF THE PREFERRED EMBODIMENT

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that  
5 no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the described device, and such further applications of the principles of the invention as illustrated therein being  
10 contemplated as would normally occur to one skilled in the art to which the invention relates.

As previously indicated, the invention relates to improved biocidal formulations for water treatment applications based on an improved method of stabilizing  
15 metals such as copper, silver, zinc, etc. The invention employs ionic polymeric stabilizing agents, preferably in conjunction with a conventional organic sequestering agent such as alpha-hydroxyacids and the like, to sequester elemental metal cations. This formulation stabilizes metals  
20 even in alkaline environments and prevents their loss due to precipitation, thereby increasing the effective life of the biocide and preventing staining of swimming pool surfaces.

In one aspect of the present invention the metal is provided as a soluble or insoluble metal salt such as metal  
25 acetates, metal chlorides, metal formates, metal nitrates, metal sulfates, or metal carbonates. The metal may also be present in its elemental form. The amount of metal salt present may vary from 0.01% to 99.9% by weight as a single metal salt or mixed metal salt composition. Preferably, the  
30 metal salt is present in an amount of about 1% to 40% by weight of the composition. The amount of elemental metal sequestered in the formulation may range from 0.01% to 30%, with 1% to 10% being preferred, and 3% to 8% being most preferred.



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In some preferred embodiments, the invention uses water-soluble anionic polymers such as polyacrylic acid, polymethacrylic acid, polyvinyl sulfonic acid, polystyrene sulfonic acid, polymaleic acid, polyaspartic acid, 5 polyphosphino carboxylic acids, copolymers, terpolymers, or tetrapolymers thereof or the sodium, potassium, calcium salts or metal salts of said polymers. In other preferred embodiments, water-soluble cationic polymers such as poly[oxyethylene-(dimethylimino) ethylene-(dimethylimino) 10 ethylene dichloride], polyethylene imine, dialyl dimethyl ammonium chloride, polyacrylamide derivatives, polyamines, polyvinyl amine, chitosan, polyethylene amine or a polymer of 1,6-hexanediamine-N,N,N',N'-tetramethyl or the fluoride, chloride, or bromide salts thereof, and the like are used. 15 The amount of polymer or mixture of polymers present in the composition may vary from 0.01% to 99.9% by weight; however, 10% to 50% is preferred.

The molecular weight of the ionic polymeric agent is preferably between about 500 Da and 20,000,000 Da, with 20 polymers having a molecular weight of between about 1,000 Da and 5,000,000 Da being more preferred, and polymers having a molecular weight of between about 1,000 Da and 1,500,000 Da being most preferred.

In one aspect of the invention, conventional organic 25 sequestering agents are used with the polymeric agent. For example, hydroxy-carboxylic acids, aminocarboxylic acids, polyamines, alkanolamines, polyphosphates, phosphonic acids, crown ethers, amino acids, etc. may be used in conjunction with the polymeric agent as described below. In certain 30 preferred embodiments organic acids such as, e.g., oxalic acid, suberic acid, acetic acid, tricarballic acid, succinic acid, malonic acid and maleic acid, and the salts thereof, are used. Especially preferred are the hydroxy-carboxylic acids such as, e.g., citric acid, gluconic acid, tartronic 35 acid, lactic acid, tartaric acid, malic acid, glyceric acid,

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or tetrahydroxy succinic acid, and the salts thereof, and the lactone or ester forms of such acids. The organic acids and hydroxy-acids are preferred in certain embodiments because the other noted sequestering agents are sources of nitrogen and phosphorous, two critical nutrients for algae growth. These conventional sequestering agents are typically added to the composition at 0.01% to 99.9% by weight; however, 5% to 40% is preferred.

Optionally, other components like dyes, perfumes, stabilizers, etc. can be included in the formulas described herein.

#### Example 1

##### Preparation of a copper composition with an anionic polymer and a conventional organic sequestering agent.

Forty grams of a 50% solution of partially neutralized (10 to 50 percent) polyacrylic acid was combined with 25 grams of anhydrous citric acid, 15 grams of water and 20 grams of copper sulfate pentahydrate. This formulation was heated and stirred to allow the copper sulfate to go into solution. This formulation yields a thick royal blue liquid that contains 5.1% elemental copper.

#### Example 2

##### Preparation of a copper composition with an anionic polymer and a conventional organic sequestering agent.

Forty grams of a 50% solution of a copolymer of maleic anhydride and styrene sulfonate, 20 grams of glucono-delta-lactone and 22 grams of copper acetate are heated and stirred with 18 grams of water to yield a very dark blue liquid formulation that contains 7.8% elemental copper.

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Example 3Preparation of a copper composition with a cationic polymer and a conventional sequestering agent.

Thirty-five grams of ethylene-diamine tetraacetic acid  
5 disodium salt (EDTA) is reacted with 13.5 grams of copper  
carbonate and 41.5 grams water. Some carbon dioxide is  
released in this reaction. 30 grams of a 50% solution of a  
polymer of 1,6-hexanediamine-N,N,N',N'-tetramethyl chloride  
salt is added. This formulation will yield a thick blue  
10 liquid that contains 5.8% elemental copper.

Example 4Preparation of a copper composition with an anionic polymer and a conventional organic sequestering agent.

Forty grams of a 40% solution of 2-Propenoic acid polymer  
15 with 2-hydroxy-3-(2-propenyloxy)-1-propanesulfonic acid  
monosodium salt (a copolymer of acrylic acid and an allyloxy,  
hydroxypropyl sulfonate), 20 grams of gluconodeltalactone and  
28 grams of copper sulfate pentahydrate are heated and  
stirred with 8 grams of potassium hydroxide and 4 grams of  
20 water to yield a very dark blue liquid formulation that  
contains 7.1% elemental copper.

Example 5Preparation of a copper composition with an anionic polymer.

25 Fifty grams of a 40% solution of 2-Propenoic acid polymer  
with 2-hydroxy-3-(2-propenyloxy)-1-propanesulfonic acid  
monosodium salt (a copolymer of acrylic acid and an allyloxy,  
hydroxypropyl sulfonate), 12 grams of hydrochloric acid and

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25 grams of copper sulfate pentahydrate are heated and stirred with 13 grams of water to yield a very dark blue liquid formulation that contains 6.4% elemental copper.

Example 6

- 5     Preparation of a copper composition containing a mixture of high and low molecular weight polyanions and a conventional sequestering agent.

10     Twenty-five grams of a 40% solution of 2-propenoic acid, polymer with 2-hydroxy-3-(2-propenyloxy)-1-propanesulfonic acid monosodium salt, a copolymer of acrylic acid and an allyloxy, hydroxypropyl sulfonate, 18 grams  
15     glucono-deltalactone, 15 grams of polyacrylic acid (molecular weight 1,000,000 to 1,500,000 Da) sold under the trade name (Aquatreat® AR-7H), 4 grams potassium hydroxide and 26 grams of copper sulfate pentahydrate are heated and stirred with 12 grams of water to yield a very dark blue liquid formulation that contains 6.6% elemental copper.

Example 7

- 20     Preparation of a dry mixed metal composition containing a polymer salt combined with a conventional sequestering agent.

25     Seventy-four and one-half grams of copper sulfate pentahydrate, 60 grams of citric acid, 60 grams of sodium polyacrylate, 5 grams of zinc sulfate, 1 gram of silver nitrate. This mixture is stirred using a dry component mixing device such as a V blender. This mixture is packaged as is or compressed into a stick or puck to change the dissolution characteristics.

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Example 8Preparation of a copper composition with a high molecular weight cationic polymer and a conventional sequestering agent.

Forty-two grams of triethanolamine is reacted with 28  
5 grams of copper sulfate pentahydrate and 25 grams of water.  
Five grams of a dialyl dimethyl ammonium chloride high  
molecular weight cationic polymer is added to the formula.  
This formula gives a very dark blue solution containing 7.1%  
copper.

10

Example 9Preparation of a silver composition with an anionic polymer.

Fifteen grams of a 40% solution of 2-propenoic acid  
polymer with 2-hydroxy-3-(2-propenyloxy)-1-propanesulfonic  
15 acid monosodium salt (a copolymer of acrylic acid and an  
allyloxy, hydroxypropyl sulfonate), and 2 grams of silver  
nitrate are diluted in 83 grams of water to give a clear  
yellow solution that has 1.3% elemental silver.

Example 10

20 Preparation of a zinc composition with an anionic polymer  
and a conventional sequestering agent.

Twenty grams of a 25% solution of salt (a copolymer of  
maleic anhydride and styrene sulfonate), 2 grams citric acid  
and 5 grams of zinc acetate are diluted in 80 grams of water  
25 to give a light yellow solution that has 1.8% elemental zinc.

Examples 11-13Biocidal effectiveness.

Minimum inhibitory concentration (MIC) laboratory studies  
were performed to demonstrate that the compositions of the  
30 present invention retained their biocidal efficacy. MIC data

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gives the minimum concentration of biocide needed to prevent growth of the algae in a culture.

The MIC studies are performed in sterile glassware using both viability and sterility controls. An algae suspension is added to test tubes containing the appropriate amounts of test biocide and the tubes are incubated for two weeks under fluorescent lights (on a "12 hour on"/"12 hour off" lighting regimen). The cultures are then measured by visual observation to determine the presence of living algae.

As seen in Examples 11-13, there was equivalent biocidal activity between free elemental copper as copper sulfate, copper complexed with triethanolamine ("TEA"), and the polymer sequestered copper at equivalent doses of active copper present against various blue-green and green algal species.

#### Example 11

MIC results against Phormidium sp. using various concentrations of copper.

Copper Conc. (ppm)	0.50	0.25	0.125	0.065	0.025
Unsequestered					
Copper	-	-	-	-	-
TEA Sequestered					
Copper	-	-	-	-	-
Polymer Sequestered					
Copper	-	-	-	-	-

(-) indicates no algae growth was seen  
(+) indicates algae growth was seen

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Example 12

MIC results against Phormidium inudatum using various concentrations of copper.

	Copper Conc. (ppm)	3.0	1.0	0.5	0.25	0.125
5	Unsequestered Copper	-	-	-	-	-
	TEA Sequestered Copper	-	-	-	-	-
	Polymer Sequestered Copper	-	-	-	-	-

10 (-) indicates no algae growth was seen  
 (+) indicates algae growth was seen

Example 13

MIC results against Chlorella sp. using various concentrations of copper.

15	Copper Conc. (ppm)	10.0	9.0	8.0	7.0	6.0
	Unsequestered Copper	-	-	-	-	-
	TEA Sequestered Copper	-	-	-	-	-
20	Polymer Sequestered Copper	-	-	-	-	+

(-) indicates no algae growth was seen  
 (+) indicates algae growth was seen

Example 1425 Field tests.

Further tests were done using test swimming pools infested with algae. These pools were treated with either the polymer sequestered copper or copper triethanolamine at a concentration of 0.7 to 0.5 ppm. All the pools treated with the formulation of the invention killed the algae; however some of those pools treated with the copper triethanolamine complex failed to kill the algae. The polymer additive may, unexpectedly, effectively "deliver" the copper to the cell

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wall, thus bringing higher concentrations of the copper in closer contact with the cell. This may be a mechanism to explain the result because copper triethanolamine works by simple diffusion.

#### Example 15

5

#### Residence time of copper.

Soluble elemental copper is easily removed from solution through filtration and by combining with carbonate, chloride or hydroxide ions that are commonly found in process water, such as swimming pools, spas or industrial water applications to form insoluble copper carbonates and hydroxides. Sequestered metals do not readily form these insoluble salts or filter out of the system. Thus, sequestered metals have a higher residence time in the water. This allows the metal to work for longer periods of time.

10  
15

The residence time of copper was tested using a system of aquaria. Copper was tested in various sequestered forms and as free soluble copper. Initially, the copper concentration will be 1.25 ppm as  $\text{Cu}^{++}$ . Copper concentrations were tested photometrically using a HACH 3000 colorimeter.

20

Initial copper concentrations were tested 15 minutes after the addition of granular or liquid ingredients. Copper concentrations were checked at 24 hours and 48 hours. The volume of the aquarium is 28 liters. The experimental design tested a copper sulfate only control, a copper triethanolamine (TEA) complex, and the polymer-copper. See FIG. 1.

25



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Example 16Residence time in field tests.

Residence times were tracked in field pools that were treated with either copper sequestered with TEA or copper sequestered with polymer. The tests show equivalent residence times for those samples containing copper/TEA and copper/polymer formulations. See FIGS. 2 & 3.

Examples 17-20Biocidal effectiveness.

Minimum inhibitory concentration (MIC) laboratory studies were performed to demonstrate that the compositions of the present invention retained their biocidal efficacy. MIC data gives the minimum concentration of biocide needed to prevent growth of bacteria in a culture.

Individual MIC's were determined by screening on microtiter plates. In order to determine the MIC's, 50  $\mu$ l of phosphate water is added to each row in columns 2-12. Next, 100  $\mu$ l of a biocide is added to column 1 (A-H). Fifty microliters of the biocide is removed from column 1 and serially diluted from columns 2-11 using an eight tip pipettor (octapette). Finally, 50  $\mu$ l of microbial inoculum is added to columns 1-11 (all rows) and column 12 (row A and E only). Column 12 serves as the row for sterility and viability controls.

Bacterial inocula contain about  $1 \times 10^9$  colony forming units (cfu) per ml are prepared in 1/5 strength Nutrient broth. Bacterial microtiter plates are incubated at 37°C for 48 hours. MIC's are determined by visual observation of the plates.

As seen in Examples 17-20, there was equivalent biocidal activity between free elemental silver and the polymer sequestered silver at equivalent doses of active silver.

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Example 17

MIC results against E.Coli using various concentrations of silver.

	Silver Conc. (ppm)	25	12.5	3.1	1.56	0.39
	Unsequestered					
5	Silver	-	-	-	+	+
	Polymer Sequestered					
	Silver	-	-	-	+	+

(-) indicates no bacterial growth was seen

(+) indicates bacterial growth was seen

10

Example 18

MIC results against S.aureus using various concentrations of silver.

	Silver Conc. (ppm)	25	12.5	1.56	0.78	0.39
	Unsequestered					
15	Silver	-	-	-	+	+
	Polymer Sequestered					
	Silver	-	-	-	+	+

(-) indicates no bacterial growth was seen

(+) indicates bacterial growth was seen

20

Example 19

MIC results against E.Coli using various concentrations of copper.

	Copper Conc. (ppm)	50	25	12.5	1.56	0.78
	Unsequestered					
25	Copper	-	-	+	+	+
	Polymer Sequestered					
	Copper	-	-	+	+	+

(-) indicates no bacterial growth was seen

(+) indicates bacterial growth was seen

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Example 20

MIC results against S.aureus using various concentrations of copper.

5	<u>Copper Conc. (ppm)</u>					
	25	12.5	1.56	0.78	0.39	
Unsequestered						
Copper	-	-	-	+	+	
Polymer Sequestered						
Copper	-	-	-	+	+	

(-) indicates no bacterial growth was seen  
 (+) indicates bacterial growth was seen

- 10 While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the
- 15 invention are desired to be protected.

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## CLAIMS

What is claimed is:

1. A biocidal composition for treating water,  
comprising:
  - (a) an biocidally effective source of a member  
5 selected from the group consisting of copper, silver, zinc,  
and mixtures thereof; and
  - (b) a polymeric sequestering agent.
2. A biocidal composition according to claim 1 wherein  
10 said polymeric sequestering agent is a water-soluble anionic  
polymer.
3. A biocidal composition according to claim 1 wherein  
said polymeric sequestering agent is a water-soluble cationic  
polymer.
4. A biocidal composition according to claim 1 and  
15 further including an organic sequestering agent differing in  
composition from said polymeric sequestering agent.
5. A biocidal composition according to claim 2 wherein  
said water-soluble anionic polymer is a member selected from  
the group consisting of polyacrylic acid, polymethacrylic  
20 acid, polyvinyl sulfonic acid, polystyrene sulfonic acids,  
polymaleic acid, polyaspartic acid and polyphosphino  
carboxylic acid.
6. A biocidal composition according to claim 5 wherein  
said water-soluble anionic polymer is a copolymer,  
25 terpolymer, or tetrapolymer of one or more of said  
water-soluble anionic polymers.

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7. A biocidal composition according to claim 5 wherein said water-soluble anionic polymer is provided as a salt.

8. A biocidal composition according to claim 3 wherein said water-soluble cationic polymer is a member selected from the group consisting of poly[oxyethylene-(dimethylimino) ethylene-(dimethylimino) ethylene dichloride], polyethylene imine, dialkyl dimethyl ammonium chlorine, polyacrylamide derivatives, polyamines, polyvinyl amine, chitosan, polyethylene amine and polymers of 1,6-hexanediamine-N,N,N'-tetramethyl.

9. A biocidal composition according to claim 5 wherein said water-soluble cationic polymer is a copolymer, terpolymer, or tetrapolymer of one or more of said water-soluble cationic polymers.

10. A biocidal composition according to claim 8 wherein said water-soluble cationic polymer is provided as a salt.

11. A biocidal composition according to claim 1 wherein said polymer is present in an amount of between about 10% and about 50%, by weight of the composition.

12. A composition according to claim 1 wherein said polymeric sequestering agent has a molecular weight of between about 500 Da and about 20,000,000 Da.

13. A composition according to claim 12 wherein said polymeric sequestering agent has a molecular weight of between about 1,000 Da and about 5,000,000 Da.

14. A composition according to claim 13 wherein said

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polymeric sequestering agent has a molecular weight of between about 1,000 Da and about 1,500,000 Da.

15. A method of reducing the ability of metal-containing biocides to stain pool surfaces, comprising providing the  
5 metal-containing biocide to a pool in a form in which at least some of the metal is sequestered by a polymeric sequestering agent.

16. A method according to claim 15 wherein said polymeric sequestering agent is a water-soluble anionic  
10 polymer;

17. A method according to claim 15 wherein said polymeric sequestering agent is a water-soluble cationic polymer.

18. A method according to claim 15 wherein said biocidal  
15 composition further includes an organic sequestering agent differing in composition from said polymeric sequestering agent.

19. A method according to claim 15 wherein said water-soluble anionic polymer is a member selected from the  
20 group consisting of polyacrylic acid, polymethacrylic acid, polyvinyl sulfonic acid, polystyrene sulfonic acids, polymaleic acid, polyaspartic acid and polyphosphino carboxylic acid.

20. A method according to claim 16 wherein said  
25 water-soluble anionic polymer is a copolymer, terpolymer, or tetrapolymer of one or more of said water-soluble anionic polymers.

21. A method according to claim 16 wherein said

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water-soluble anionic polymer is provided as a salt.

22. A method according to claim 17 wherein said water-soluble cationic polymer is a member selected from the group consisting of poly[oxyethylene-(dimethylimino) ethylene-(dimethylimino) ethylene dichloride], polyethylene imine, dialyl dimethyl, ammonium chloride, polyacrylamide derivatives, polyamines, polyvinyl amine, chitosan, polyethylene amine, polymers of 1,6-hexanediamine-N,N,N',N'-tetramethyl.

23. A method according to claim 17 wherein said water-soluble cationic polymer is provided as a salt.

24. A method according to claim 15 wherein said polymer is present in an amount of between about 10% and about 50%, by weight of the composition.

25. A method according to claim 15 wherein said polymeric sequestering agent has a molecular weight of between about 500 Da and about 20,000,000 Da.

26. A method according to claim 25 wherein said polymeric sequestering agent has a molecular weight of between about 1,000 Da and about 5,000,000 Da.

27. A method according to claim 26 wherein said polymeric sequestering agent has a molecular weight of between about 1,000 Da and about 1,500,000 Da.

28. A method extending the useful life of a metal-containing biocide, comprising providing the metal-containing biocide to a pool in a form in which at least some of the metal is sequestered by a polymeric sequestering agent.

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29. A method according to claim 28 wherein said polymeric sequestering agent is a water-soluble anionic polymer.

5 30. A method according to claim 28 wherein said polymeric sequestering agent is a water-soluble cationic polymer.

31. A method according to claim 28 wherein said biocidal composition further includes an organic sequestering agent differing in composition from said polymeric sequestering agent.  
10

32. A method according to claim 29 wherein said water-soluble anionic polymer is a member selected from the group consisting of polyacrylic acid, polymethacrylic acid, polyvinyl sulfonic acid, polystyrene sulfonic acids, polymaleic acid, polyaspartic acid and polyphosphino carboxylic acid.  
15

33. A method according to claim 30 wherein said water-soluble cationic polymer is a member selected from the group consisting of poly[oxyethylene-(dimethylimino) ethylene-(dimethylimino) ethylene dichloride], polyethylene imine, dialyl dimethyl, ammonium chloride, polyacrylamide derivatives, polyamines, polyvinyl amine, chitosan, polyethylene amine, polymers of 1,6-hexanediamine-N,N,N',N'-tetramethyl.  
20

25 34. A method according to claim 28 wherein said polymer is present in an amount of between about 10% and about 50%, by weight of the composition.

35. A method according to claim 28 wherein said



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polymeric sequestering agent has a molecular weight of between about 500 Da and about 20,000,000 Da.

36. A method according to claim 35 wherein said  
polymeric sequestering agent has a molecular weight of  
5 between about 1,000 Da and about 5,000,000 Da.

37. A method according to claim 36 wherein said  
polymeric sequestering agent has a molecular weight of  
between about 1,000 Da and about 1,500,000 Da.

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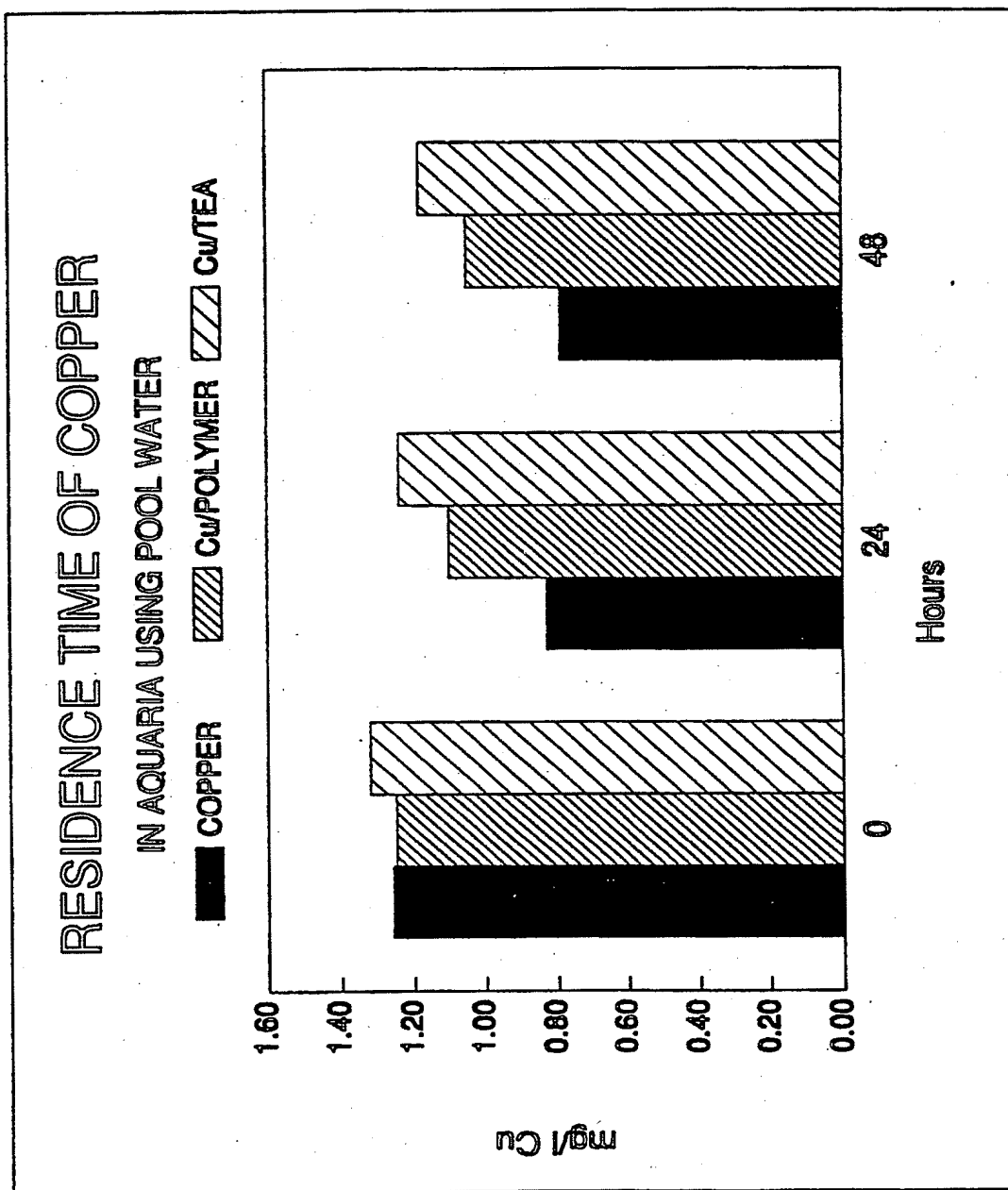


Fig. 1

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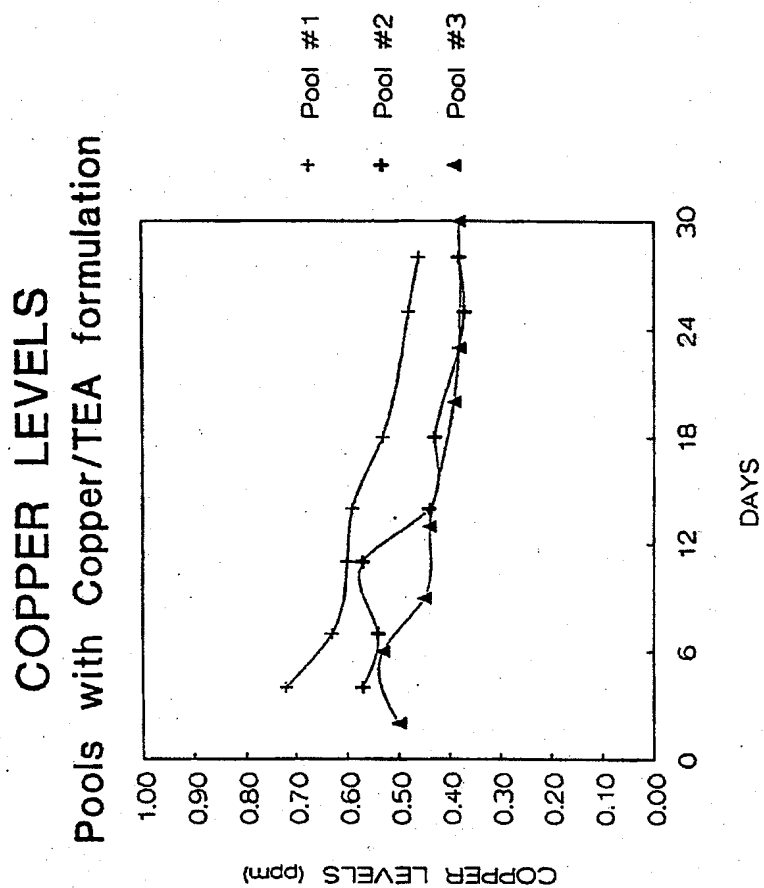


Fig. 2

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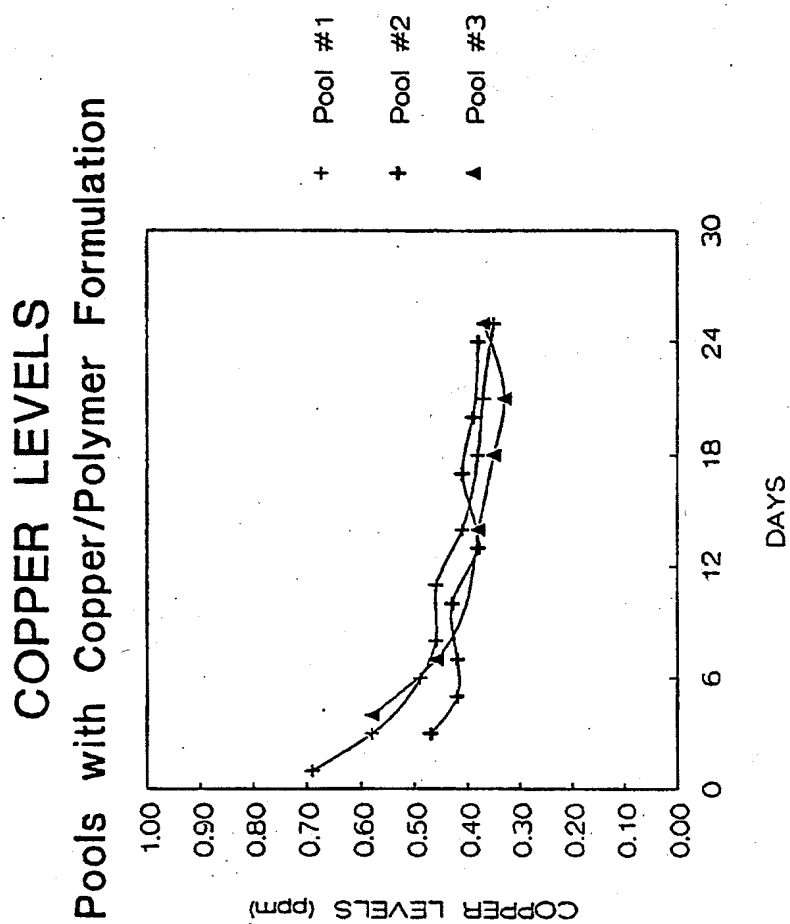


Fig. 3

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/07872

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01N 55/02, 59/20

US CL : 504/151, 152, 157

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 504/151, 152, 157

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS

search terms: COPPER, ALGICID?, SEQUESTER?, POLYMER?, POLYACRYLIC, POLYVINYL

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,324,477 (SCHROEDER AT AL) 28 June 1994.	1-37
A	US, A, 4,324,578 (SEYMOUR ET AL) 13 April 1982.	1-37
A	US, A, 5,160,527 (LAW ET AL) 03 November 1992.	1-37
A	US, A, 5,149,354 (DELANEY) 22 September 1992.	1-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*Z\* document member of the same patent family

Date of the actual completion of the international search

22 AUGUST 1996

Date of mailing of the international search report

17 SEP 1996

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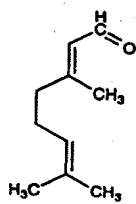
BRIAN BEMBENICK

Telephone No. (703) 305-4551

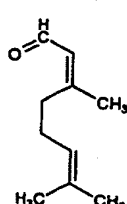
Form PCT/ISA/210 (second sheet)(July 1992)\*

## Citramalic Acid

Separation of isomers: Naves, *Bull. Soc. Chim.* 1952, 521. Comprehensive reviews: J. L. Simonsen, *Terpenes*, vol. I, 83-100 (1947); E. Guenther, *The Essential Oils*, vol. II, 326-336 (1949); Gildemeister-Hoffmann, *Aetherischen Oele*, vol. IV, 307-336 (4th ed., 1956).



Geranial (Citral a)



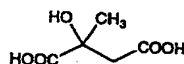
Neral (Citral b)

Geranial, light oily liquid. Strong lemon odor.  $bp_{25}$  92-93°.  $d_4^{20}$  0.8888.  $n_D^{20}$  1.48982. Practically insol in water. Miscible with alc, ether, benzyl benzoate, diethyl phthalate, glycerol, propylene glycol, mineral oil, essential oils.

Neral, light oily liquid. Lemon odor not as intense but sweeter than geranial.  $bp_{25}$  91-92°.  $d_4^{20}$  0.8869.  $n_D^{20}$  1.48690. Solubilities same as geranial.

USE: In the synthesis of vitamin A, ionone and methylionone. As a flavor, for fortifying lemon oil. In perfumery for its citrus effect in lemon and verberna scents, in cologne odors, in perfumes for colored soaps. Not stable to alkalis and strong acids. Will cause discoloration of white soaps and alkaline cosmetics.

2384. Citramalic Acid. 2-Hydroxy-2-methylbutanedioic acid; 2-methylmalic acid; 2-hydroxy-2-methylsuccinic acid;  $\alpha$ -hydroxypyrotartaric acid.  $C_5H_8O_5$ ; mol wt 148.12. C 40.55%, H 5.44%, O 54.01%. Enzymatic synthesis: Barker, Blair, *Biochem. Preps.* 9, 21 (1962). Chemical synthesis: Barker, *ibid.* 25; J. B. Wilkes, R. G. Wall, *J. Org. Chem.* 45, 247 (1980). Stereoselective synthesis: E. G. J. Staring *et al.*, *Rec. Trav. Chim.* 105, 374 (1986).

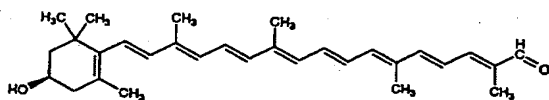


*dl*-Form, deliquescent monoclinic prisms from ethyl acetate + petr ether, mp 117°. Sublimes. Freely sol in water, acetone. Sol in ethyl acetate, ether. Practically insol in petr ether, benzene.

*d*-Form, crystals, mp 112.2-112.8°.  $[\alpha]_D^{25} +23.6^\circ$  ( $c = 3$  in  $H_2O$ ).

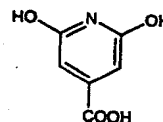
*l*-Form, crystals, mp 112-113°.  $[\alpha]_D^{25} -23.4^\circ$  ( $c = 3$  in  $H_2O$ ).

2385.  $\beta$ -Citaurin. 3-Hydroxy-8'-apo- $\beta$ , $\psi$ -carotenal; citaurin.  $C_{30}H_{48}O_2$ ; mol wt 432.65. C 83.29%, H 9.32%, O 7.40%. Carotenoid pigment found only in orange peel. Isoln by chromatography: Zechmeister, Tuzson, *Ber.* 69, 1878 (1936); 70, 1966 (1937). The peels from 100 kilos of oranges yield about 35 mg. Structure: Zechmeister, Tuzson, *loc. cit.*; Karrer, Solmsen, *Helv. Chim. Acta* 20, 682 (1937); Karrer *et al.*, *ibid.* 1020; Zechmeister, v. Chohnoky, *Ann.* 530, 291 (1937); Karrer *et al.*, *Helv. Chim. Acta* 21, 445 (1938). Abs config: Bartlett *et al.*, *J. Chem. Soc. (C)* 1969, 2527. Synthesis: H. Pfander *et al.*, *Helv. Chim. Acta* 63, 1377 (1980).



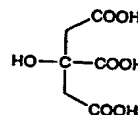
Thin orange or yellow-colored plates from benzene + petr ether, mp 147°. Absorption max (benzene): 497, 467 nm. Freely sol in acetone, ethanol, ether, benzene, and carbon disulfide. Sparingly sol in petr ether.

2386. Citrazinic Acid. 1,2-Dihydro-6-hydroxy-2-oxo-4-pyridinecarboxylic acid; 2,6-dihydroxyisonicotinic acid; 2,6-dihydroxy-4-pyridinecarboxylic acid.  $C_6H_5NO_4$ ; mol wt 155.11. C 46.46%, H 3.25%, N 9.03%, O 41.26%. Prep'n from citric acid with aq  $NH_3$  at 140-160° under pressure: Bavyly, Hamilton, U.S. pat. 2,729,647 (1956 to Pfizer). Purification: Bavyly *et al.*, U.S. pat. 2,738,352 (1956 to Pfizer).



Yellowish powder with a greenish tinge; carbonizes above 300° without melting. Ultrapure material which is white or colorless, has been prepared. Almost insol in water; slightly sol in hot HCl; sol in alkali hydroxide or carbonate solns. Alkaline solns turn blue on standing.

2387. Citric Acid. 2-Hydroxy-1,2,3-propanetricarboxylic acid;  $\beta$ -hydroxytricarballic acid.  $C_6H_8O_7$ ; mol wt 192.13. C 37.51%, H 4.20%, O 58.29%. Widely distributed in plants and in animal tissues and fluids. Produced by mycological fermentation on an industrial scale using crude sugar solns, such as molasses and strains of *Aspergillus niger*: See review by Von Loesecke, *Chem. & Eng. News* 23, 1952 (1945); Schweiger, U.S. pat. 2,970,084 (1961 to Miles Labs.); Faith, Keyes & Clark's *Industrial Chemicals*, F. A. Lowenheim, M. K. Moran, Eds. (Wiley-Interscience, New York, 4th ed., 1975) pp 275-279. Also extracted from citrus fruits (lemon juice contains 5 to 8%) and from pineapple waste. Reviews: Wilson, *Chem. & Met. Eng.* 29, 787 (1923); Browne, *Ind. Eng. Chem.* 13, 81 (1921); Warneford, Hardy, *ibid.* 17, 1283 (1925); E. F. Bouchard, E. G. Merritt in Kirk-Othmer *Encyclopedia of Chemical Technology* vol. 6 (Wiley-Interscience, New York, 3rd ed., 1979) pp 150-179. Toxicity: Gruber, Halbeisen, *J. Pharmacol. Exp. Ther.* 94, 65 (1948).



Anhydr form, mp 153°. Crystals are monoclinic orthorhombic and crystallize from hot concd aq soln. d 1.665. At 25°,  $pK_1$  3.128;  $pK_2$  4.761;  $pK_3$  6.396, Bates, Pinching, *J. Am. Chem. Soc.* 71, 1274 (1949). Soly in water: 54.0% w/w at 10°; 59.2% at 20°; 64.3% at 30°; 68.6% at 40°; 70.9% at 50°; 73.5% at 60°; 76.2% at 70°; 78.8% at 80°; 81.4% at 90°; 84.0% at 100°.

Monohydrate, orthorhombic crystals from cold aq solns. Pleasant, sour taste. d 1.542. Monohydrate crystals lose water of crystn in dry air or when heated at about 40 to 50°, slightly deliquescent in moist air. Softens at 75°. mp ~100°. pH of 0.1N soln = 2.2. Densities of aqueous soln (15°/15°): 10% = 1.0392; 20% = 1.0805; 30% = 1.1244; 40% = 1.1709; 50% = 1.2204; 60% = 1.2738. Soly in g/100 g satd soln: ether 2.17; chloroform 0.007; amyl alcohol 15.43; amyl acetate 5.98; ethyl acetate 5.28. Soly at 19° in g/100 g solvent: methanol 197; propanol 62.8. LD<sub>50</sub> i.p. in rats: 975 mg/kg (Gruber, Halbeisen).

Pharmaceutical Incompatibilities: Potassium tartrate, alkali and alkaline earth carbonates and bicarbonates, acetates, sulfides. Dilute aq solns may ferment on standing.

Barium salt heptahydrate,  $C_{12}H_{16}Ba_2O_{14} \cdot 7H_2O$ , barium citrate. Powder. Loses all  $H_2O$  at 150°. Sol in 1750 parts water; freely sol in dil HCl or  $HNO_3$ ; practically insol in alcohol.

Ethyl ester,  $C_{12}H_{20}O_7$ , ethyl citrate, triethyl citrate. Bitter, oily liq.  $d_4^{20}$  1.137.  $bp_{100}$  294°;  $bp_{15}$  127°. Viscosity at 25°: 35.2 cps. Pour pt ~10°.  $n_D^{20}$  1.4455. Soly: water ~6.9% peanut oil 0.8%. Misc with alc, ether.

USE: Acidulant in beverages, confectionery, effervescent salts, in pharmaceutical syrups, elixirs, in effervescent powders and tablets, to adjust the pH of foods and as syn-

gistic antioxi-  
jellies, jams,  
manuf of alk  
inhibitor. In  
agent to rem  
colors; in ele  
istry for det  
albumin, mu  
THERAP CA  
(citrate dextro  
acid syrup).

2388. Citri-  
trimethyl-6-oxo-  
cin.  $C_{13}H_{14}O_5$   
31.97%. Antit  
aspergillus whi  
Aspergillus nive  
small quantitie  
Raistrick, Tran  
trick, Smith, C  
nin, *Science* 96,  
*Health* 35, 80  
*Arch. Pharm.* 2  
*Chem. Soc.* 194  
3812 (1957); Kc  
sis: Cartwright  
ber *et al.*, *J. Che*  
chemistry: Crar  
ta, Whalley, J. C  
*ibid.* 1964, 464  
Nagai *et al.*, *Che*  
Crystal and mol  
1971, 1553. Bi  
*Perkin Trans. I*  
Physicochemical  
*Chem. Soc.* 54, 2219  
Eds, *J. Pharmac*  
*et al.*, "Yellowed  
ler, S. Kadis, A  
1971) vol. VI, pp

Lemon-yellow  
-37.4° ( $c = 1.15$   
418). Strong acid.  
hol, dioxane, dilute  
in pH, from lemon  
9.9. Poisonous! 1  
(Ambrose, De Eds.  
Methyl citrinin,  
 $[\alpha]_D^{25} +217.1^\circ$  ( $c =$   
( $E$ )<sub>254</sub> 520, 151.6).  
chloroform. Practi  
Dihydrocitrinin,  
171°.  $[\alpha]_D^{25} -18.8^\circ$   
260, 330 nm ( $E$ )<sub>254</sub>  
roform; sparingly sol

2389. Citromy-  
4H,5H-pyrano[3,2-  
quentic acid.  $C_{14}H_{16}O_6$   
3.47%, O 38.59%.  
cillium frequentans  
by: *Citromyces* spp;  
*Roy. Soc. London*,  
*Nature* 167, 995 (1951)  
*Chem. Soc.* 1951, 2  
1958, 4576; Money,  
sis: M. Yamauchi *et*  
395.

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Whitehouse Station, NJ

1996